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ANALYTICAL TECHNIQUES IN ENVIRONMENTAL CHEMISTRY

Proceedings of the International Congress, Barcelona, Spain, November 1978

Edited by

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Foreword

Environmental chemistry has achieved great popularity during this decade as a consequence of our increasing concern over the quality of life. However, the gathering of vital data in this field is difficulted by the complexity and wide scope of its greatly interrelated aspects on several research areas, all of them deserving serious consideration. For instance, any national or global policy making in this regard requires the previous acquisition of fundamental and applied knowledge on problems such as the worldwide distribution and interaction of chemicals, both of natural and anthropogenic origin, their modes of transport and ultimate fate as well as the effects on ecosystems, notably on biological species. In any case, the identification of toxic substances in air, water, foods and soils will continue being a constant driving force behind many outstanding research programs and progress along these lines will depend largely on the development and implementation of sensitive and reliable analytical methodologies.

The present book contains most of the papers presented at the International Congress on Analytical Techniques in Environmental Chemistry, held in Barcelona in november 1978 and organized by the Societat Catalana de Ciències Físiques, Químiques i Matemàtiques and Expoquimia. The main subject of the Congress was the result of a coincidence of interests on the part of these two organizations. On one hand, the old concern of the Societat Catalana de Ciències for a better knowledge of our environment, which had its roots in the pionering meteorological studies undertaken in the early 30's, during the International Solar Year, and on the other,

the interest of Expoquimia in reflecting through the International Chemical Exhibition the increasing efforts of the chemical industries to minimize as much as possible the environmental impact.

The selection of the topics discussed rested on the Scientific Committee to whose members (E. Casassas, G. Eglinton, M. Gassiot, E. Gelpí, G. Guiochon, R. Hites, O. Hutzinger, A. Liberti, J. Oró and J. Ros) I would like to express my personal gratitude, also extended to the many scientists which contributed with the quality of their presentations. Thanks are also due to Pergamon Press, who by taking charge of the publication of the book, has facilitated the wider possible diffusion of this information within the scientific community.

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Environmental Chemistry - An Interdisciplinary Subject. Natural and Pollutant Organic Compounds in Contemporary Aquatic Environments

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ABSTRACT

Contemporary aquatic environments generate and receive organic compounds which are of both natural and pollutant origin. The waters and sediments contain a wide range of compounds, free and bound as insoluble debris. For example, extractable lipids are present in sediments in amounts varying from ppm to a few per cent. The various component classes - hydrocarbons, fatty acids, alcohols, etc. - can each show distributions characteristic of the different types of aquatic environment. Of particular interest are the hydrocarbons, which occur ubiquitously but vary in their structural type (straight-chain, branched-chain, acyclic isoprenoid, cyclic isoprenoid, polycyclic aromatic hydrocarbons, etc.), their degree of unsaturation (alkanes, alkenes, aromatics) and their carbon numbers (typically 10-45). The hydrocarbon 'fingerprints' represented by the relative abundances of the individual members of each structural type can be correlated with known inputs and associated diagenetic effects. Specific parameters can be used to recognise natural and anthropogenic inputs and distinguish between the variety of pollutant sources. As an example, analysis of the hydrocarbons extracted from particulate fractions of Severn Estuary tidal mud shows that the 'sand', 'silt' and 'clay' fractions, separated by deflocculation and sedimentation, possess different contents of alkane and polynuclear aromatic hydrocarbons (PAH). The natural input of higher plant alkanes comprises a greater proportion of the sand-sized particles whereas the unresolved complex mixture (UCM) of branched/cyclic alkanes, the steranes and the triterpanes, which all derive from oil pollution, are more abundant in the clay-sized fraction. In contrast, the PAH, mainly derived from combustion of fossil fuels, are present in greatest proportion in the 'sand' fraction. These results show that lipids of differing origin are concentrated in different size particles of Severn Estuary mud.

Keywords: Alkanes, PAH, UCM, Lipids, Particle-size fractionation, Environmental parameters.

INTRODUCTION

Environmental chemistry is the study of the chemistry of natural environments with, of course, particular interest relating to man's influence. It encompasses the distribution of both organic and inorganic components in the geosphere, hydrosphere, atmosphere and biosphere. The interactions between organic and inorganic components in the environment are undoubtedly extensive and complex but are much less studied than either major field. This paper will

deal only with the organic components, citing an illustrative cross-section of references rather than a definitive bibliography.

Organic compounds are ubiquitous in natural environments and are present in the gaseous state, in solution and as colloids, particulate matter and organisms - all as part of the carbon cycle of this planet. Most environmental and organic compounds of natural origin are ultimately derived from biosynthesised organic compounds. At the molecular level in the environment these may be: (i) unchanged, (ii) incorporated into insoluble organic matter by chemical bonding, adsorption, trapping etc., (iii) partially altered or broken down, but retaining structural or other similarity with their biosynthesised precursor, (iv) altered to the extent that they bear little resemblence to their parent molecule, e.g. after extensive thermal treatment, or (v) completely broken down to carbon dioxide or methane. These transformations can be accomplished by both biological and non-biological (physico-chemical) means. The main difficulties in assigning a past history or ultimate origin for such organic compounds are as follows: (i) a single compound may be contributed from a multitude of sources, (ii) individual molecules from a variety of inputs may follow different chemical, physical and biological pathways to the same product compounds, (iii) a particular compound may give rise to several different products, and (iv) the fate of the molecule is dependent on time, since transformations vary from rapid (i.e. of the order of days, as in a water column) to slow (i.e. of the order of millions of years, as during diagenesis and maturation in the earth's crust).

The background of organic compounds in a given sediment is comprised of autochthonous and allochthonous components. The autochthonous input is, in part, of a direct biological origin, coming from organisms as intact biolipids, etc., and in part, of an indirect biological origin, including the microbial, chemical and geochemical alteration products generated within the water column and sediment. The sources of allochthonous inputs are more varied: they may be non-biogenic (e.g. the products of forest fires) or derived from the weathering and erosion of ancient sediments that are thermally immature (e.g. shales and brown coals), or thermally mature (e.g. oil seeps and coals). In addition, there are anthropogenic inputs from naturally occurring sources (e.g. oils and coals), although the composition of such material is often modified by refining, burning, etc., and from synthetic manufacture (e.g. DDT). The pollutant, biogenic and other natural inputs to aquatic sediments are shown schematically in Fig.1. The organic compounds from the various inputs often possess specific characteristics that betray their origin, namely their: (i) structures, (ii) stereochemistries, (iii) relative abundances, (iv) isotopic content, and (v) sites of occurrence, including depth profiles.

ORGANIC COMPOUNDS IN RECENT AQUATIC SEDIMENTS

The organic matter of Recent sediments is comprised of both solvent-inextractable and solvent-extractable organic components. The inextractable organic material is primarily composed of fragments of biopolymer, humic material and other macromolecular debris. The solvent-extractable or lipidic component consists of a variety of compound classes, notably alkanes, alkenes, polycyclic aromatic hydrocarbons (PAH), carboxylic acids, hydroxy-carboxylic acids, ketones, alcohols (especially sterols) and amino acids. The organic matter is variously contributed by autochthonous and allochthonous sources or generated *in situ* within the sediment. Usually, only a minor proportion of the organic

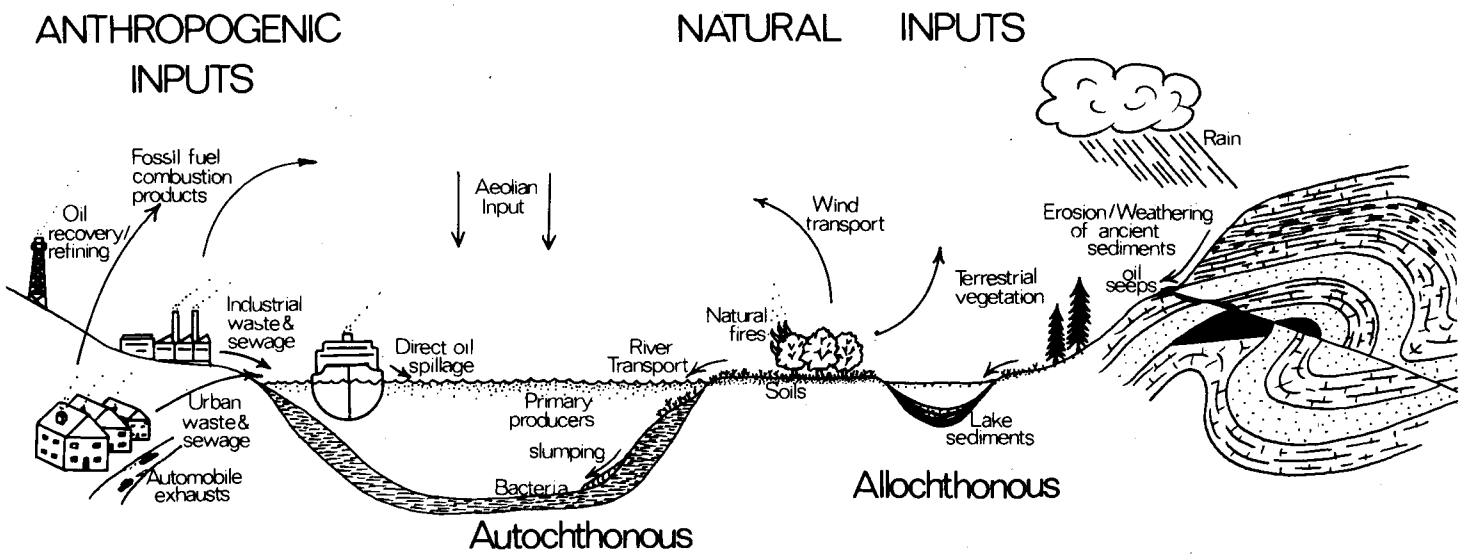


Fig.1. Pollutant and biogenic and other natural inputs of organic compounds to aquatic sediments.

This sketch illustrates sources of organic compounds and their routes of transport to aquatic sediments. The major anthropogenic inputs are shown to the left of the scheme. These pollutants include the products from combustion of fossil fuels, inputs of sewage, industrial waste, oil spillage, etc. The autochthonous and allochthonous natural inputs are given in the middle and to the right of the diagram, respectively. The autochthonous contributions to sediments include inputs from primary producers, especially phytoplankton, zooplankton, and also the bacteria inhabiting the water column and sediment. Allochthonous contributors of organic matter include both thermally mature (e.g. oil seeps) and immature (e.g. shales, brown coals) sources of indirect input. In addition, contributions from terrestrial vegetation and soils, which act as a reservoir of organic matter, comprise an integral part of the allochthonous component. Combustion products of natural origin, such as those generated by forest fires or spontaneous burning of oil shales, oil seeps, etc., are further contributors of allochthonous organic compounds. In the environment, allochthonous and pollutant organic matter is transported by a variety of mechanisms, especially potamic and aeolian means. Slumping, and in some instances, ice-rafting, can also be important agents in carrying organic matter.

matter contributed to or generated within an aquatic environment actually reaches and becomes incorporated into the underlying sediment. The major portion is selectively recycled within the water column by a wide variety of processes, including photo-oxidation, evaporation, dissolution, particle association, predation and microbial degradation. Microbial activity is of key importance, especially as it occurs within the water column, in animal guts and faeces and continues in the sediment, contributing thereby anabolic, catabolic and metabolic products and gross cellular debris. The organic compounds that reach the sediment and escape degradation by the indigenous macrobial and microbial hierarchy may remain as 'free' lipids or undergo processes such as absorption, adsorption, inclusion and incorporation that lead to the early-stage precursor of kerogen: 'protokerogen'.

The processes mentioned above are a part of a wider biogeochemical cycle that involves inorganic carbon (e.g. carbonate), organic compounds and organisms. A key role is played by phytoplankton and other photosynthetic organisms that use light, inorganic nutrients and carbon dioxide to produce the bulk of the autochthonous organic matter that feeds the zooplankton and supports the complex aquatic food web. The associated macro- and microorganisms generate the rain of organic-rich debris that descends through the water column to the underlying sediment. Thus, the nature of the water column and the bottom sediment greatly influences the extent and type of preservation of organic matter within sedimentary environments. In particular, the oxicity/anoxicity conditions appear to be crucial, although they are themselves, determined by many inter-related parameters, including the rate of sediment accumulation, the level of organic productivity and the topography of the depositional basin, in so far as it influences water circulation and hence nutrient supply. In general, an oxic water column and underlying bottom sediment (e.g. continental shelves) result in a poor preservation of organic matter both in quantitative terms and at the molecular level. By degrees, the extent of preservation improves in moving towards a mainly anoxic water column and bottom sediment (Didyk et al., 1978), as seen in the present-day Black Sea. Organic productivity in the photic zone is dependent on the nutrient supply. Thus, Walvis Bay, off the coast of Namibia, a region supplied with Si, N and P from the polar regions of the South Atlantic by the Benguela current, is an area of high productivity. The sediments beneath the shallow waters of this portion of the African Continental Shelf, periodically receive massive inputs of biological debris, such as decaying diatom blooms (Hart and Currie, 1960). Their organic carbon content is therefore high and rich in lipidic material, well-preserved by the induced anoxicity and highly-reducing conditions consequent upon such inputs. Walvis Bay is a natural marine reducing environment (Eisma, 1969): contemporary, man-induced counterparts are eutrophic lakes, where a high level of organic pollution exists or where high biological productivity has been caused by pollutant inputs.

Within the sediment, the fate of the organic matter is again influenced by chemical, physical and biological processes. In particular, the redox conditions and acidity play a major role in determining the nature and rate of diagenetic reactions, both directly and indirectly via their effect on the bacterial population and, conversely, their effect on the microenvironment within the sediment. Most sediment depth profiles may be divided into oxic, intermediate and reducing zones (Fenchel and Reidl, 1970) populated by a hierarchical sequence of macro- and microorganisms. As the extent of degradation of organic matter is greatest within oxic environments, the rate at which it passes through the upper sediment horizons or is buried by further deposition

must significantly affect its degree of preservation, especially as bacteria are concentrated at the sediment/water interface (Zobell, 1964).

There are several ways in which the origin and fate of organic compounds in a given environment can be evaluated:
First, survey methods can be used to obtain a general picture of the organic content of a localised environment by determining the components of the organisms (such as the species of plants surrounding or living in a lake) contributing to particular sediments (Nishimura and Koyama, 1977; Giger and Schaffner, 1977). Such a study can be conducted on a geographical basis by sampling within a specific area, or historically by investigating organic profiles with sediment depth; for example, determining the onset of the flux of pollutant hydrocarbons derived from man's combustion of fossil fuels (Farrington et al., 1977a; Farrington and Tripp, 1977; Boehm and Quinn, 1978). The magnitude of such tasks increases in proportion to the size of the chosen area or the length of the sediment core, making it easier to apply such correlations to lakes and estuaries then to marine environments. In addition to whole sediment analysis, the association of individual organic species with discrete particle sizes can be investigated by size fractionation prior to analysis (Thompson and Eglinton, 1978a). This method of study has shown that certain organic compounds are concentrated in particular size fractions. The flux of organic compounds within a chosen environment can also be evaluated using sediment traps.

Second, important aspects of the marine food web can be studied. Every species of organism, not only those living within the water column, but also those inhabiting the upper layers of sediment, has a distinct niche in the hierarchy of the food web. The overall complexity of the interactions and relationships between the organisms precludes a complete investigation of such systems, even relatively simple ones such as algal-bacterial mats. A specific segment of the web can, however, be selected, studied and evaluated. For example, at Bristol, the constituents of copepod faecal pellets are being investigated so as to reveal the degradation and alteration processes acting on the phytoplankton lipids that constitute the zooplankton diet. Such a study requires laboratory cultures of the chosen species of organisms and appropriate feeding experiments (Volkman et al., unpublished data).

Third, the short-term fate of organic compounds in Recent sediments can be investigated directly by laboratory and/or field incubations of selected 'marker' compounds (Javor et al., 1979). Normally such studies are carried out over periods of hours to months which can be taken to correspond to the time scale of early-stage diagenetic processes. Recent investigations have included studies of algal decay (Cranwell, 1976) and the incubations of sterols and stanols under different conditions of oxicity to determine their diagenetic pathways (Nishimura and Koyama, 1977; Nishimura, 1978). The products generated from the chosen precursor can be traced most conveniently by using radiolabelled substrates. The activities and half-lives of ^{14}C and ^{3}H make these isotopes suitable labels for such studies (Brooks and Maxwell, 1974; Gaskell and Eglinton, 1975; Gaskell et al., 1976; de Leeuw et al., 1977a and b). Alternatively, an unlabelled compound can be incubated (Nishimura and Koyama, 1977; Nishimura, 1978) in quantities sufficient to dominate the eventual analytical results. Ideally such investigations should be performed with a minimum of disturbance to the environment under study so that the validity of the results as an accurate reflection of the natural system is preserved (Javor et al., 1979).

CHARACTERISATION OF MIXTURES OF ORGANIC COMPOUNDS EXTRACTED FROM NATURAL ENVIRONMENTS.

Full molecular characterisation is essential to the proper description of lipids and other organic compounds extracted from natural environments. However, several parameters are especially valuable in relating compounds to possible sources:
First, stereochemical data are useful to the environmental chemist because most organisms biosynthesise specific stereoisomers which may undergo epimerisation into more thermodynamically stable configurations when subjected to elevated temperatures during diagenesis and maturation. The stereochemistry of a particular compound may therefore reflect its diagenetic history (Patience et al., 1978; Mulheirn and Ryback, 1975; Ensminger et al., 1977), so that inputs from organisms and Recent and ancient sediments can be distinguished. In addition, the stereochemistry of the diagenetic products can reveal whether or not particular diagenetic reactions are stereospecific and thereby assist in defining such reactions as biological or physico-chemical (Brooks et al., 1978).

Second, homologous series of organic compounds are commonly the result of biosynthesis and often survive in geological samples. Many species of organisms biosynthesise series of straight-chain compounds (e.g. n-alkanes, n-fatty acids, and n-alcohols) by the process of carbon chain elongation with acetate units. The process is not held precisely to a fixed number of units, thereby producing a series of dominant members that differ by two carbon numbers. Bacteria and some species of diatoms are notable exceptions in that their n-alkanes do not show a dominance of alternate carbon numbers within the homologous series biosynthesised. Diagenetic processes modify the concentrations of individual members of an homologous series, although the series itself may survive, even to extreme levels of sediment maturity or microbial degradation. The relative concentrations of an homologous series, such as the n-alkanes, can therefore be a reflection of its origin and maturity. The presence of homologous series in biological systems and mature sediments and oils can be conveniently investigated by mass fragmentography in C-GC-MS analyses utilising the fact that the individual members possess common ions in their mass spectra; for example, all n-alkanoic acid methyl esters give m/e 74 as the base peak.

Third, in addition to homologous series, natural and polluted systems give rise to pseudohomologous series, such as acyclic and polycyclic isoprenoid alkanes. These series comprise compound classes that possess common structural features, for example, all hopanes possess the same pentacyclic triterpenoid skeleton, differing only in the length of their alkyl side chains and stereochemistries. Like homologous series, the distribution of individual pseudohomologous series members is dependent on their source (e.g. the range of alkylated PAH present in the combustion products of fossil fuels is more limited than that found in mature sediments and oils: compare Laflamme and Hites, 1978 with Brassell et al., in press, and Speers and Whitehead, 1969). Mass fragmentography of key ions (e.g. m/e 217 for steranes; Leythaeuser et al., 1977; Seifert, 1977 and 1978; Seifert and Moldowan, 1978 and 1979), is again a convenient means of rapid recognition in C-GC-MS analyses.

Fourth, organisms synthesise characteristic carbon number ranges of homologous and pseudohomologous series. This feature is often preserved in aquatic environments, except where extensive bacterial alteration has taken place or in instances where the natural inputs have been swamped by pollutants. Indeed,

pollutant inputs can be recognised by their masking of the biological alkane characteristics. The differences in biological carbon number ranges are valuable in chemotaxonomic classifications (Eglinton et al., 1962; Eglinton and Hamilton, 1963), and enable environmental interpretations to be made from sedimentary lipid distributions (Brooks et al., 1976 and 1977; Cranwell, 1977). For example, the n-alkanes synthesised by algae generally fall in the C_{15} to C_{21} range, and are dominated by n-C_{17} (Oró et al., 1967; Gelpi et al., 1970; Blumer et al., 1971) whereas higher plants typically produce odd-numbered n-alkanes in the C_{23} to C_{37} range and upwards (Eglinton et al., 1962; Caldicott and Eglinton, 1973). Such variations in these values result from the different functions of these alkanes in the respective plant species. Their preservation in aquatic sediments furnishes valuable information about biological inputs.

Fifth, the carbon preference index (CPI) is a further tool used to assess and distinguish between different biological contributions to sediments. In addition, it can aid the recognition of pollutant inputs. For n-alkanes, the CPI is defined as the ratio of the quantity of odd to even chain length components, specified for a given carbon number range (Cooper and Bray, 1963). As a general rule, CPI decreases with increasing sediment maturity, tending to unity, a value typical of most, but not all, oils (Bray and Evans, 1961). Many species of biota show considerable carbon preference in the range of their biosynthesised straight-chain components, principally alkanes, carboxylic acids, alcohols and ketones. This intrinsic feature of photosynthetic organisms is a result of the biochemical process of chain elongation. There are, however, exceptions to the simplistic model that the CPI tends to unity with increasing maturity because certain classes of organism, bacteria being an important example, do not show a prominent carbon preference in their lipid composition (Han et al., 1968). When considered with the indications of other data, such ambiguities of interpretation are usually clarified. Given these provisos, CPI remains a valuable indicator of the maturity of sediment lipid contributions, distinguishing natural inputs (CPI of alkanes generally high) from pollutant sources (CPI of alkanes roughly unity).

Sixth, the modality of lipid distributions is another useful source indicator. Thus, the lipids of marine flora and higher plants possess significantly different carbon number ranges and their combined inputs give rise to bimodal distributions; for example, twin carbon number maxima with low (C_{15}-C_{18}) and high (C_{25}-C_{31}) values in the case of n-alkanes. However, the great majority of petroleums possess unimodal n-alkane distributions with a maximum at low carbon number (e.g. n-C_{16}, Martin et al., 1963; Tissot and Welte, 1978), as a result of carbon chain shortening and contributions from the cracking of kerogen during the processes of diagenesis and maturation. Since pollutant inputs of alkanes are principally derived from petroleums or fossil fuels of similar maturity, they are also characterised by a maximum at low carbon number, although this will be influenced by evaporation, volatilisation and selective microbial degradation (Brassell et al., 1978). Unimodal distributions are often indicative of a single type of source of organic matter, whereas bimodal, trimodal or greater distributions suggest mixed sources.

Seventh, isotopic information enables crude assessment of the sources of organic matter in an environment. In this respect, $\delta^{13}C$ values can distinguish between terrestrial and marine components of organic matter (Hedges and Parker, 1976), allowing allochthonous and autochthonous inputs to be evaluated. The range of $\delta^{13}C$ values of the major sources of pollutants is usually insufficient to enable recognition of the precise origin (i.e. whether from an oil spillage or from fossil fuel combustion fallout) of this component of the organic matter

because of the dilution of such inputs by the natural component. $\delta^{13}C$ data provide an overall, averaged picture of a given environment rather than specific details on individual aspects.

The most versatile analytical method for the evaluation of the various parameters discussed above, with the exception of isotopic and detailed stereochemical data, is computerised gas chromatography-mass spectrometry (C-GC-MS). The necessary ability to handle the complex mixtures encountered in environmental analyses is ably provided by C-GC-MS, and at the sub-nanogramme level. An example of the utility of this technique is given later in this paper. In addition to C-GC-MS, capillary gas chromatography can provide comparative analyses of the volatile components of complex mixtures, while high pressure liquid chromatography (HPLC) is suitable for investigations of labile or less volatile compounds.

LIPID INDICATORS OF THE ORIGIN AND DIAGENESIS OF SEDIMENTARY ORGANIC MATTER.

Sedimentary lipids can provide an indication of the source of the organic matter in aquatic environments by their identity with known biosynthetic compounds. In addition, a significant proportion of geolipids can be recognised as the diagenetic products of biolipid precursors (e.g. sterenes and steranes from sterols) and are thereby attributable to possible inputs of organic matter. There are, however, difficulties in associating geolipids and original sources, particularly the fact that many geolipids and their postulated biolipid precursors have not been reported in organisms. For example, Henrichs and Farrington (personal communication) have shown that the range of free amino acids in the interstitial water of marine sediments includes many that can be assigned to biological inputs, but there are other major components present which have not been so related. There is also the problem of assessing the natural background levels of organic compounds for a particular environment which existed prior to man's activities. For example, this is a major problem in connection with the widespread contemporary combustion of fossil fuels. Thus, the worldwide presence of anthropogenic polynuclear aromatic hydrocarbons (PAH) makes it difficult to assess the natural background levels of these compounds, generated by biological or other precursors, such as forest fires, which are thought to have made a signficant contribution to sediments in the geological past (Youngblood and Blumer, 1975).

Chirality is an important feature of many lipids, as organisms often biosynthesise a single stereoisomer which could possess one or many chiral centres. As already mentioned, such stereoisomers may undergo epimerisation over geological time, initially during diagenesis by the action of physicochemical conditions and biochemical agents, and subsequently during maturation by thermal processes. The transformation of isoleucine to alloisoleucine is an example of a geologically rapid epimerisation, occurring in the order of 10^5-10^7 years dependent on microenvironment (Bada and Schroeder, 1972). In particular, the epimerisation of acyclic isoprenoid alkanes, steranes and hopanes is a slower process, usually occurring over 10^6-10^8 years at elevated temperatures, although these stereochemical changes can be simulated in laboratory studies by still higher temperatures in the order of days or months (Eglinton, 1972; Connan, 1974). Fig.2 illustrates the stereochemical features of the hopanoids, where three positions, C-17, C-21 and C-22 are of particular interest and importance. The hopanoid alkanes of immature sediments are principally the 17βH,21βH-isomers as single C-22 diastereoisomers. Smal-

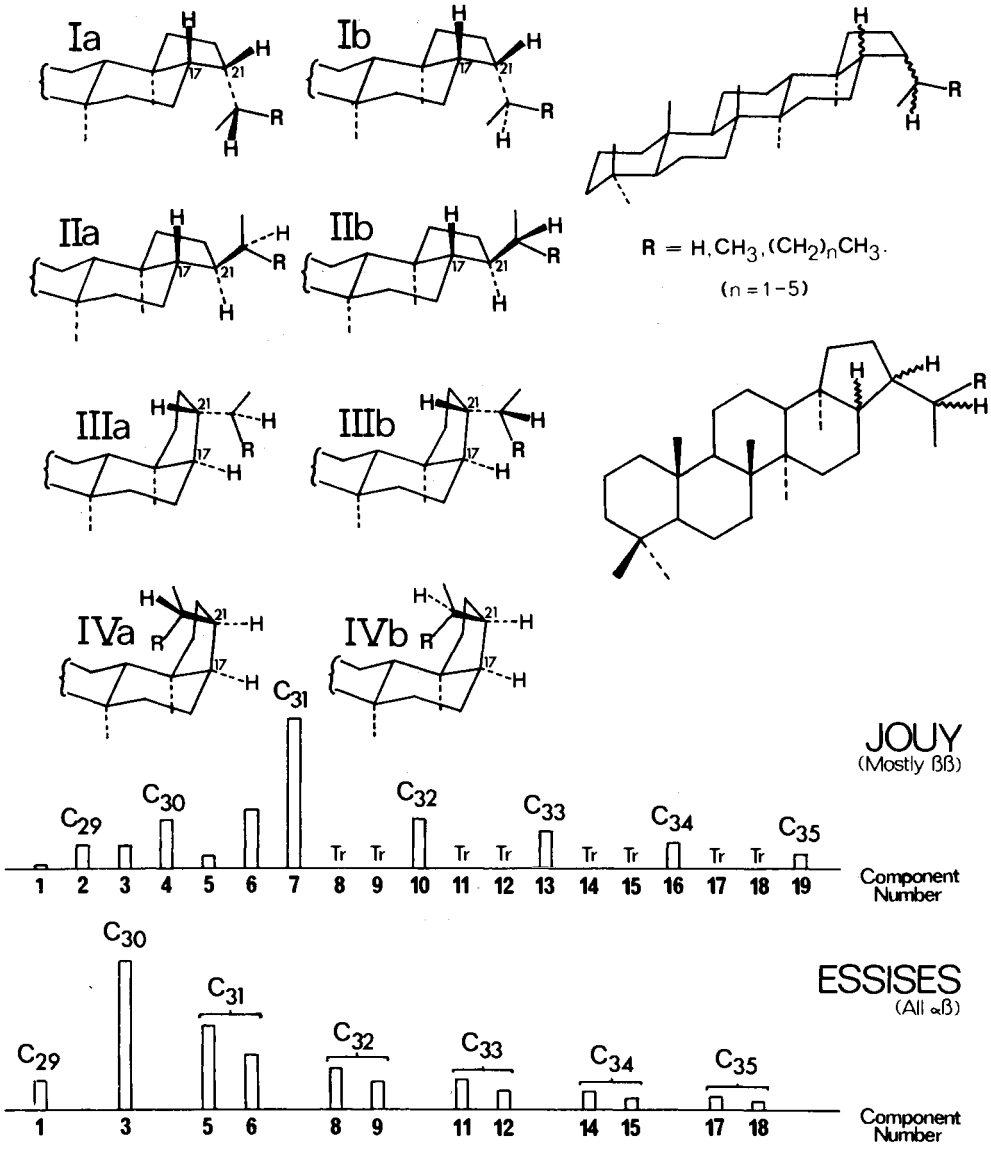


Fig.2. Extended hopanoid series (I-IV) of triterpenoids found in organisms, Recent and ancient sediments and oils.

The four types of C_{30} skeleton are hopanes (17βH or 17αH,21βH; I, III; R=Me) and moretanes (17βH or 17αH,21αH; II, IV; R=Me). With increasing maturity the more thermodynamically stable 17αH-configuration (III and IV) becomes dominant while epimerisation of the C-22 position in the extended hopanoids (R=alkyl) also occurs, but more slowly. For example, the dominant stereochemistry of each of the extended hopanoids from different horizons of the Toarcian shales of the Paris Basin show a change from a single 17βH,21βH C-22 diastereoisomer (Ia or Ib) at Jouy (700m deepest burial) to a pair of 17αH,21βH C-22 diastereoisomers (IIIa and IIIb) at Essises (2540m deepest burial) (Ensminger, 1977). (Tr indicates component present in trace quantities).

ler amounts of the 17βH,21αH-hopanes (i.e. 17βH-moretanes) as single C-22 diastereoisomers are also present, probably formed from their 17βH,21βH counterparts by clay-catalysed isomerisation (Ensminger et al., 1977; Ensminger, 1977). The process of thermal maturation effects a conversion of 17βH,21βH- and 17βH,21αH-isomers with a single C-22 configuration into C-22 diastereoisomeric pairs of the thermodynamically more stable 17αH,21βH- and 17αH,21αH-configurations, respectively. The differences in shape between compounds with the 21βH- and the 21αH-configurations results in easy separation by gas chromatography. These stereochemical transformations enable the hopanes of mature shales and petroleums to be distinguished from those of thermally-immature sediments. Inputs of mature organic matter from both natural erosion processes and anthropogenic sources can therefore be recognised in Recent sediments (Dastillung and Albrecht, 1976). The use of hopane fingerprints is a good example of the way in which stereochemical data can indicate the source of organic matter in the environment.

A ubiquitous feature of polluted sediments from all parts of the world is the presence of an unresolved complex mixture (UCM) of alkanes observed in gas chromatographic analyses (e.g. Farrington and Quinn, 1973 and 1977a; Eglinton et al., 1975; Thompson and Eglinton, 1978). The UCM is most pronounced in sediments where bacterial activity has selectively removed the n-alkanes and other readily biodegraded hydrocarbons and where the more volatile alkanes have been lost by evaporation in the environment or during sample work-up. A prominent UCM can also develop when a natural input of a seep or a weathered oil shale is modified by bacteria. The presence of a large UCM in a sediment cannot be interpreted unambiguously as an indicator of anthropogenic input. However, polycyclic components such as the hopanes are also resistant to bacterial degradation; the characteristic fingerprint of such compounds is preserved even after extensive microbial alteration and may serve to distinguish between possible inputs to an aquatic environment. Similiar considerations apply to the suite of polycyclic aromatic hydrocarbons (PAH) introduced into the environment as the products of fossil fuel combustion (Lao et al., 1973; Thompson and Eglinton, 1978b; Giger and Schaffner, 1977; Müller et al., 1977; Eglinton et al., 1975; Laflamme and Hites, 1978). Specific PAH are also derived naturally from both tetracyclic and pentacyclic triterpenoids during diagenesis and maturation (Greiner et al., 1976 and 1977; Spyckerelle et al., 1977a and b; Schaefle et al., 1978). Perylene is a further example of a natural PAH of widespread occurrence in Recent sediments (Orr and Grady, 1967; Aizenshtat, 1973; Laflamme and Hites, 1978), immature ancient sediments (Simoneit and Burlingame, 1974; Barnes et al., in press; Brassell et al., in press) and oils (Carruthers and Cook, 1954). Bush fires can be expected to contribute PAH to the environment and may have been the dominant source of these components in temperate zones from the development of large areas of forested land in the Miocene to the onset of man's activity. The typical PAH composition produced by fossil fuel combustion is dominated by unsubstituted components (fluoranthene, pyrene, etc.) whereas in shales and crude petroleums alkylated compounds are the major PAH constituents (Coleman et al., 1973; Youngblood and Blumer, 1975). Inputs for these two sources can therefore be distinguished on the basis of the degree of alkylation, except that the quantities of PAH are generally small in crude petroleums and large in the products of fossil fuel combustion. A small input of combustion products can therefore partially obscure the PAH distribution of a more significant petroleum input, although analytical data for other lipids (e.g. alkanes) can often resolve such problems in the interpretation of mixed pollutant inputs.

Faecal sterols exemplify a further class of pollutant lipid encountered in

aquatic environments (Murtaugh and Bunch, 1967; Tabak et al., 1972; Dutka et al., 1974). Thus, the sediments of estuaries and inshore waters are subjected to inputs of untreated sewage, for example, the Clyde Estuary (Goodfellow et al., 1977) and the Hudson Canyon (Hatcher et al., 1977) show sterol distributions which may be used to 'map' the extent of the pollutant input.

DISTINGUISHING NATURAL AND POLLUTANT INPUTS IN THE ENVIRONMENT: AN EXAMPLE

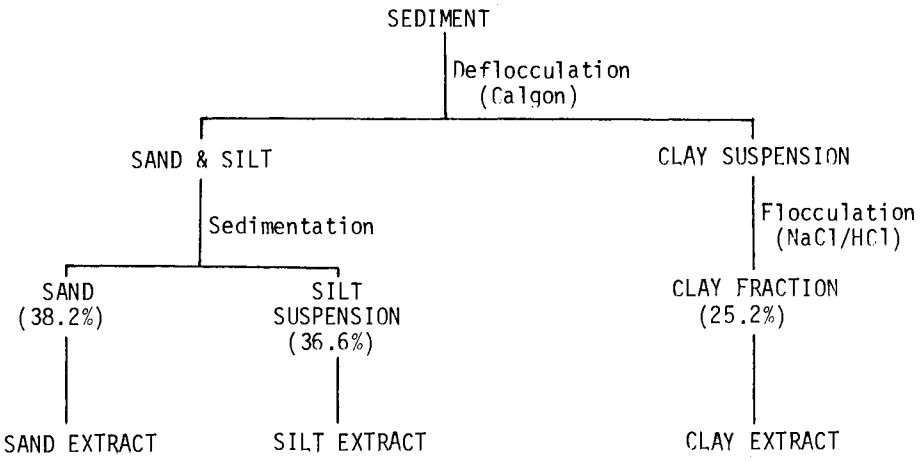
Lipid analysis of sized fractions of sediments from an English lake(Rostherne Mere, Cheshire) has shown that the compounds characteristic of higher plant inputs are indeed associated with the coarse fraction that contains visible fragments of plant debris, whereas pollutant petroleum hydrocarbons are concentrated in the finer 'clay' fraction (Thompson and Eglinton, 1978a). Oil pollution in Rostherne Mere sediment is minor in extent compared with that in the muds of the Severn Estuary, which shows massive inputs of oil and fossil fuel combustion products (Thompson and Eglinton, 1978b). To determine whether or not the pollutants present in the Severn Estuary muds are also associated with certain sizes of particle, samples of sediment have been fractionated and analysed according to the scheme given in Fig.3. The scheme produces fractions of 'sand' 'silt' and 'clay'-sized particles that are subsequently extracted. After TLC separation and urea adduction, the normal alkanes, branched/cyclic alkanes (mainly UCM) and PAH obtained from each fraction are analysed by gas chromatography (Table 1) and, in some instances, by combined gas chromatography-mass spectrometry.

TABLE 1 Total amounts (µg/g dry wt. sediment) of specific component classes separated from each particle size fraction.

		ALKANES		
Fraction	%	Straight-chain	Branched/cyclics (mainly UCM)	PAH
Sand	38.2	0.6	1.7	3.7
Silt	36.6	0.9	5.3	1.2
Clay	25.2	1.0	23.3	0.8

The relative abundances of normal alkanes in the three fractions are given in Fig.4. In qualitative terms, the relative proportions of individual members are generally similar, although the quantity of normal alkanes is significantly greater in the 'clay' fraction compared to the 'silt' and 'sand' fractions. The higher odd-numbered alkanes (especially n-C_{27}, n-C_{29} and n-C_{31}), which represent the higher plant contribution to the sediment, do not show a marked increased concentration relative to the other normal alkanes in any of the fractions. The ratio of higher plant alkanes to total alkanes is greatest in the 'sand'-sized fraction which parallels the observed trend of Rostherne Mere sediments (Thompson and Eglinton, 1978a), suggesting that higher plant material is concentrated in the coarser particulate matter. The lower n-alkanes with a CPI roughly equal to unity are indicative of oil pollutants. The UCM of aliphatic hydrocarbons appears in the gas chromatographic records for the non-adducted (branched/cyclic) alkanes of all three size fractions. There are minor qualitative differences, the main feature being the much larger quantity

(A) Fractionation and extraction of lipids.



(B) Separation of lipid extracts.

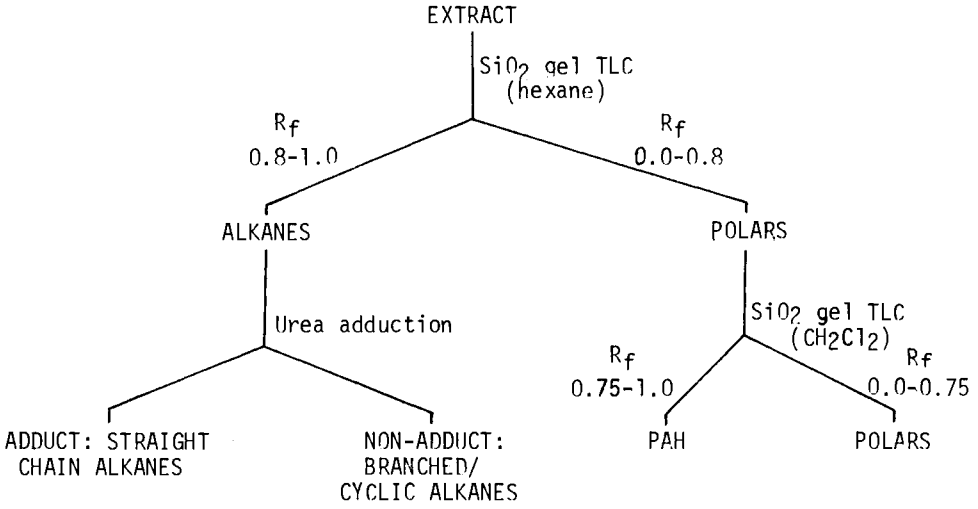


Fig.3. Schemes for (A) Fractionation and extraction of lipids and (B) Separation of lipid extracts for Severn Estuary sediments.

Sediment samples were taken at a depth of 30-45cm in the tidal mud at a site close to Aust Warth (Thompson and Eglinton, 1976). The fractionation scheme was chosen to achieve an improved separation of clay (25.2% of the total sediment) from other particle sizes, as sieving techniques without deflocculation had afforded only a minor (~1%) clay fraction. Each particle size fraction was extracted by sonication with i-PrOH/hexane (4:1). Analysis of the non-adducted and adducted fractions and PAH was performed by capillary gas chromatography and selected fractions were further evaluated by computerised gas chromatography-mass spectrometry.

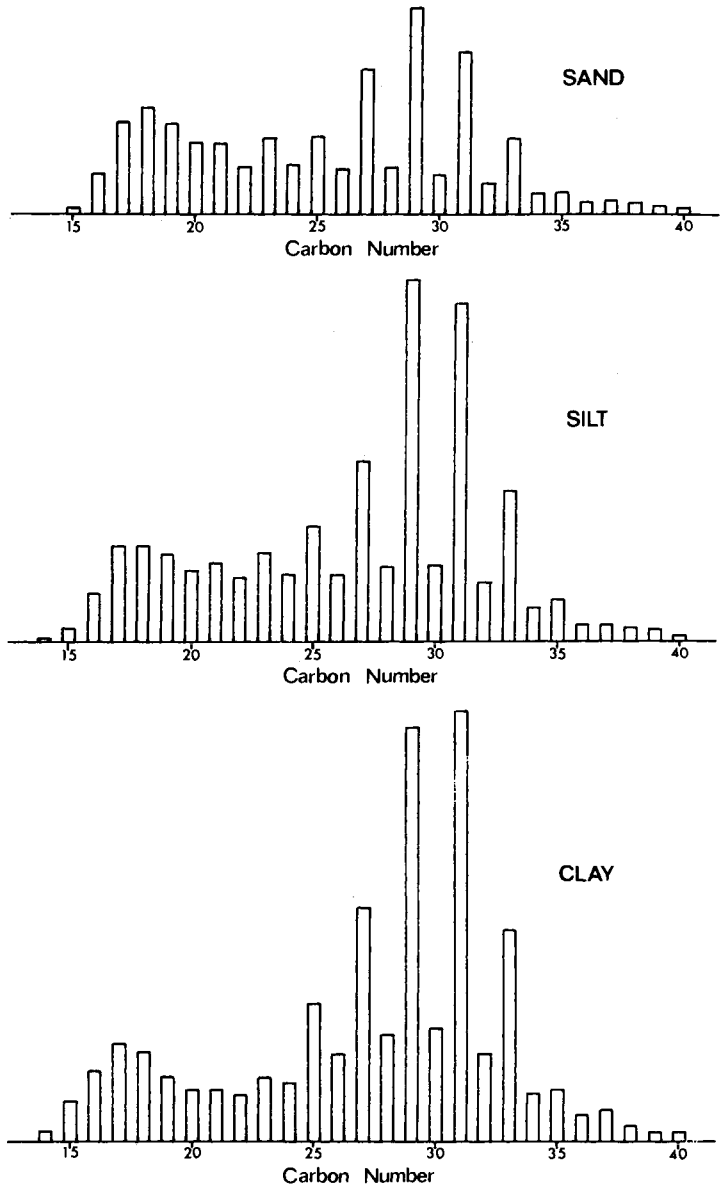


Fig.4. Normal alkane distributions in the different size fractions of Severn Estuary sediment.

Each fraction is quantitated by capillary gas chromatography (GC), with the individual amounts of n-alkanes in the sand and silt fractions shown relative to their concentration in the clay fraction (i.e. 1 µℓ injection from 500 µℓ total alkane fraction for each particle size). GC conditions: 18m SE-52 WCOT column, temperature programmed from 50^{o} to 275^{o}C at 4^{o}C/min., after splitless injection at ambient temperature. Normal alkanes from n-C_{40} to n-C_{45} are also present in trace quantities in each size fraction.

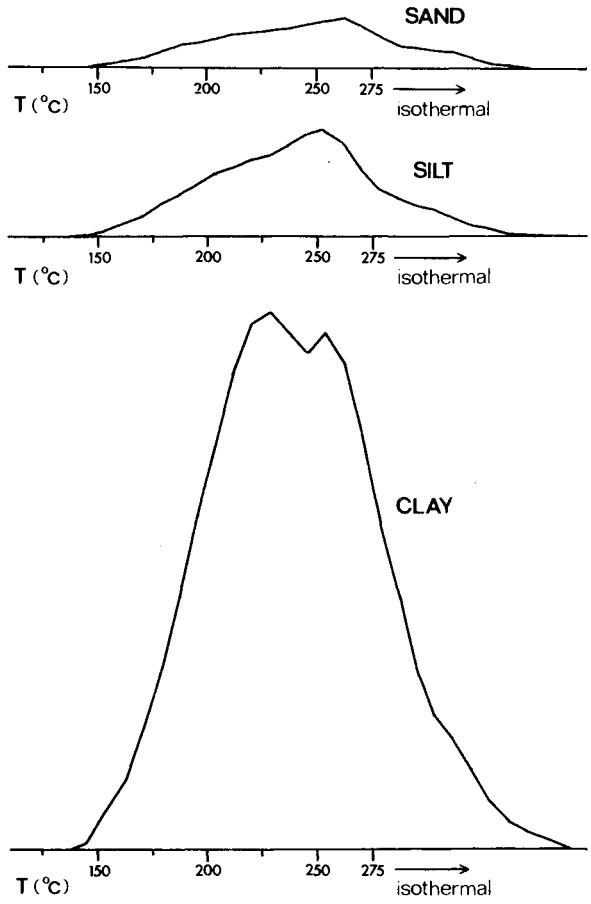


Fig.5. UCM distributions in the three size fractions, determined from capillary gas chromatography. (GC conditions and quantitation as Fig.4.).

of UCM in the 'clay' than in the 'silt' and 'sand' fractions (Fig.5). The oil pollutants that comprise this 'hump' are therefore primarily associated with clays. The size of the 'hump' also suggests that microbial degradation of the crude oil input has been extensive so that the microbes effecting this biodegradation may be predominantly associated with the 'clay' fraction.

Capillary C-GC-MS analysis of the non-adducted 'clay' fraction (Fig.6) was performed using a 20m OV-1 capillary column, temperature programmed from 50° to 260°C at 6°C/min, scanning over m/e 50-500 at 2.5s/scan. Mass spectra (35eV) were acquired from 130°C. The total ion current of the non-adducted alkanes from the clay-sized fraction also shows a large UCM, but mass fragmentography enables various classes of component within the 'hump' to be recognised from their characteristic fragment ions. Thus, the m/e 217 fragmentogram reveals the sterane distribution as a complex mixture of stereoisomers typical of mature sediments and oils (Mulheirn and Ryback, 1975; Brassell et al., 1978; Seifert, 1978; Seifert and Moldowan, 1978 and 1979) while the m/e 191 fragmentogram illustrates the diastereoisomeric hopane pairs that also characterise mature oils and sediments (Dastillung and Albrecht, 1976; Ensminger et al., 1977). These data confirm the presence of substantial proportions of pollutant aliphatic hydrocarbons of petroleum origin.

The PAH distributions (Fig.7) are similar in the three size fractions. The quantity of PAH in the 'sand' fraction, however, is an order of magnitude higher than in the 'clay' fraction. This observation can be interpreted in two ways. First, the fossil fuel combustion products that are the major PAH components may be introduced as sand-sized particulate matter. Second, solvent extraction may be less efficient in removing PAH adsorbed onto clay-sized particulates than sand-sized ones. The degree of adsorption of PAH onto montmorillonite is less than that onto suspended organic matter (Herbes, 1977) so that the clays themselves do not play the predominant role, but rather it is the clay-sized organic matter that is the most influential.

The quantities and nature of the PAH components in Severn Estuary sediment strongly support an origin from the combustion of fossil fuels rather than from direct crude oil spillage (Thompson and Eglinton, 1978b).

In summary, this fractionation of Severn Estuary sediment has shown that although the hydrocarbons of the three size fractions are qualitatively similar, they differ considerably in quantitative terms. In particular, the coarser 'sand' fraction possesses the greatest quantity of PAH, whereas the 'clay' fraction contains the majority of the UCM of alkanes in the sediment. These observations suggest that oil pollutants (e.g. the UCM) are predominantly associated with the finer particles, whereas the products from the combustion of fossil fuels, mainly PAH, are primarily associated with the coarser particles. Different sediment inputs appear therefore to be concentrated in particles of different sizes. Hence, size fractionation shows promise as a technique for the investigation of the origins of organic compounds in sediments, especially when combined with detailed microscopic examination of each fraction. In addition, simulation experiments both in the laboratory and in situ, involving the addition of specific pollutants to sediments, are likely to help reveal the kinetics and sites of association within the sediment. Sediment fractionation methods should aid in the understanding of the mutual relationships between lipids and their associated inorganic sedimentary matter just as recent investigations of inextractible and bound lipids (Farrington et al., 1977b; Nishimura, 1977; Cranwell, 1978) have increased the knowledge of early-stage diagenetic processes of lipid incorporation.

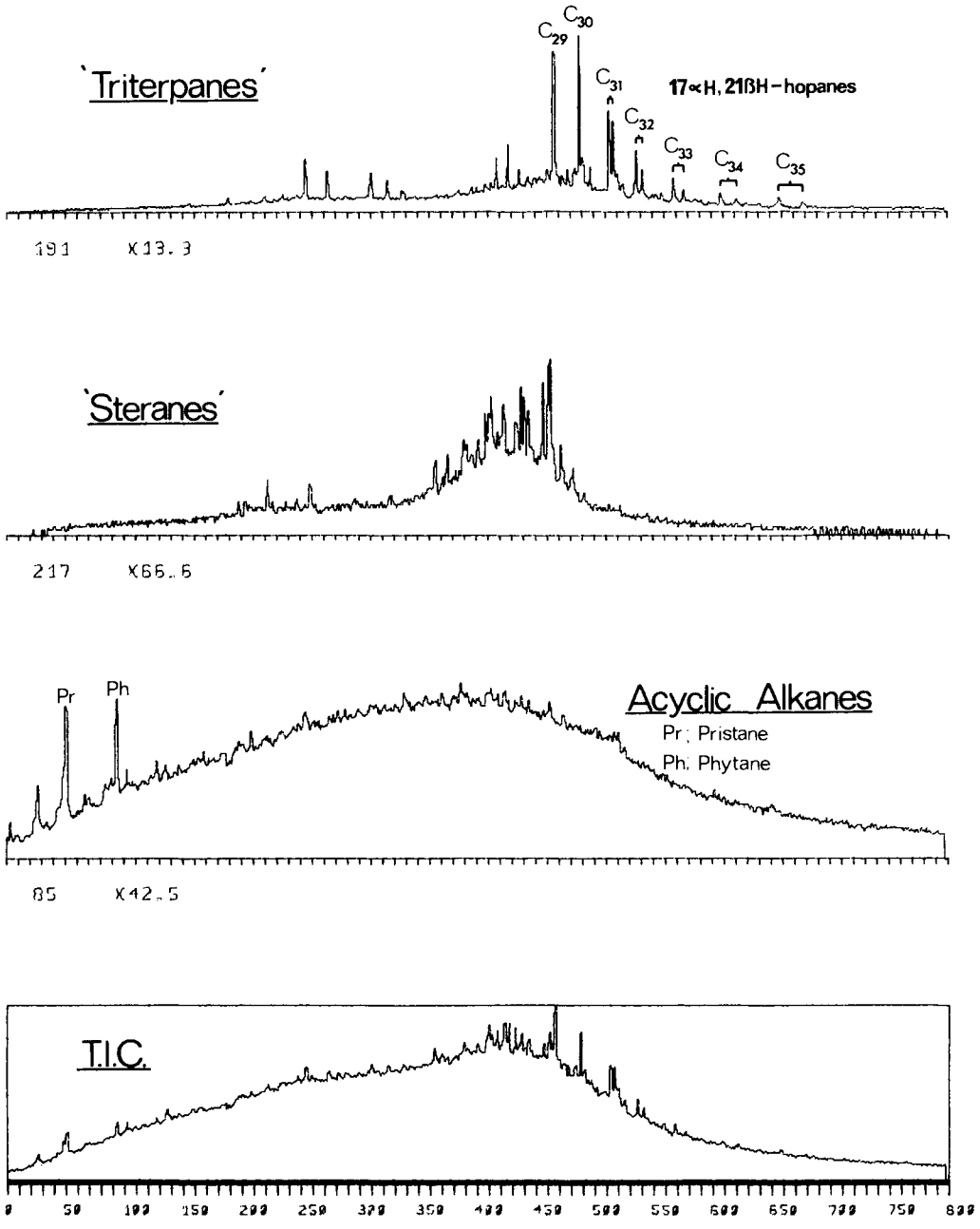


Fig.6. Selected data from C-GC-MS analysis of UCM in non-adducted 'clay' fraction from Severn Estuary sediment.

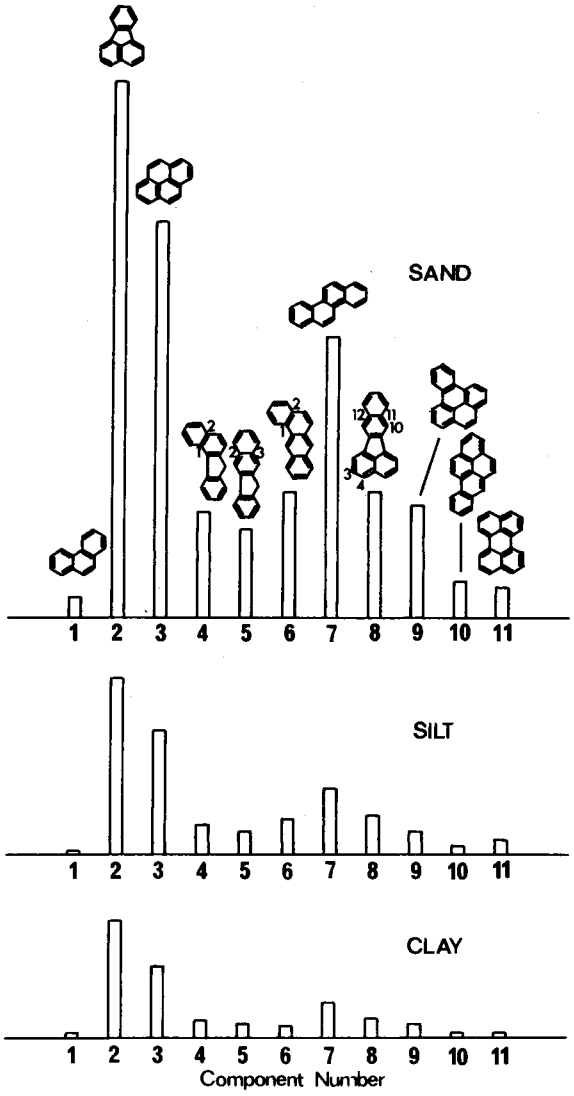


Fig.7. Distributions of prominent polynuclear aromatic hydrocarbons (PAH) present in three size fractions of Severn Estuary sediment. (GC conditions and quantitation as Fig.4).

Selected prominent components identified by C-GC-MS* - 1, phenanthrene; 2, fluoranthene; 3, pyrene; 4, 1,2-benzofluorene; 5, 2,3-benzofluorene; 6, 1,2-benzanthracene; 7, chrysene (also triphenylene); 8, 11,12-benzofluoranthene (also 10,11-benzofluoranthene. 3,4-benzofluoranthene); 9, benzo(e)-pyrene; 10, benzo(a)pyrene; 11, perylene.

* Components 1, 7, 9 and 11 identified by C-GC-MS and GC coinjection of standards: other components by C-GC-MS and GC retention times (cf. Thompson and Eglinton, 1978b).

CONCLUSIONS

The majority of environmental chemical studies are concerned with the detection of pesticides, herbicides, pollutants and other anthropogenic products, while investigations of the compounds generated from the interaction of pollutants with natural sedimentary matter have been more limited in number. In particular, the effects of trapping and adsorption of organic compounds onto mineral phases, especially clays and interactions with humic material are undoubtedly important. It seems almost inevitable that pollutants are being incorporated into the humic structures of sediments currently being deposited and that some may parallel the fate of biolipids and become an integral part of the 'protokerogen', especially as the proportion of pollutant compounds in sediments can reach levels where it is a highly significant part of the total organic matter, and must therefore influence the formation and diagenesis of the 'protokerogen'. Many pollutant classes, especially PAH, are more resistant to microbial degradation than are natural biolipids so that their impact on the processes of early-stage diagenesis is likely to be considerable. Normal extraction procedures do not address this aspect of the long-term fate of pollutants in the geosphere, although the techniques of field desorption mass spectrometry, pyrolysis mass spectrometry, NMR and ESR can be used to investigate the bound organic matter. In such analyses, the masking effect of the natural organic matter has to be taken into account. In contrast, sediments often contain anthropogenic lipids in amounts similar to their natural lipid inputs and in this respect the analysis of extractible lipids is invariably the most convenient measure of the quality and quantity of organic pollutants.

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Recognition of the Sources of Isoprenoid Alkanes in Recent Environments

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ABSTRACT

Alkanes are ubiquitous components of contemporary aquatic and terrestrial environments and derive from natural and/or pollutant sources. In many cases, a detailed consideration of the structure and stereochemistry of individual components is necessary to distinguish the sources of these components. High resolution gas chromatography and computerised gas chromatography-mass spectrometry allow determination of the structures, including stereochemistry, of certain widely-distributed alkanes in recent sediments. Three examples of such compounds have been chosen to illustrate the principles by which these structural determinations permit assignment to particular sources: (i) pristane (2,6,10,14-tetramethylpentadecane) from a lacustrine sediment is shown to derive from a mixed source (biological and pollutant); (ii) 17βH-hopane in the same sediment appears to derive from reduction of hop-22(29)-ene, an abundant alkene of Microcystis aeruginosa, present in the lake; (iii) 17α(H)-homohopane in a sphagnum peat arises mainly as a result of bacterial decay of Sphagnum cuspidatum.

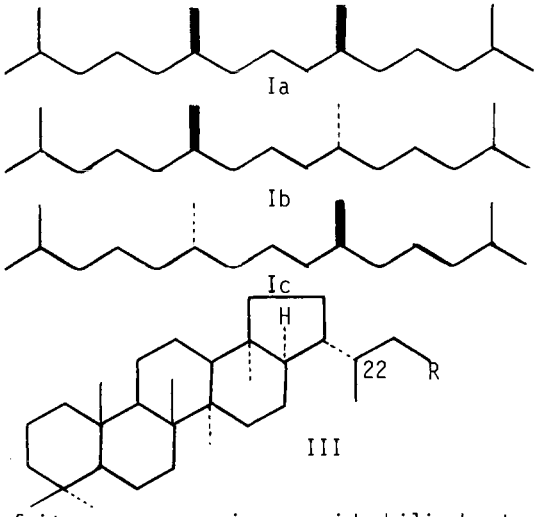
Keywords: pristane, 17β(H)-hopane, 17α(H)-homohopane, Microcystis aeruginosa, Sphagnum cuspidatum, peat, sediments.

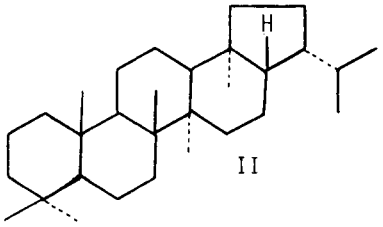
INTRODUCTION

Alkanes occur widely in recent sediments as complex mixtures readily amenable to analysis by capillary gas chromatography (GLC) and computerised gas chromatography-mass spectrometry (C-GC-MS) (e.g. Giger and Schaffner, 1978; Thompson and Eglinton, 1978). These compounds can derive from a number of sources, which include a contribution from living organisms (either directly, or indirectly by way of a precursor compound converted to the alkane in the sediment). Alkanes may derive from a variety of pollutant sources. In some cases the gross structure of an individual alkane, or the distribution of a series of alkanes, reflects a particular input. For example, the presence of 7- and 8-methylheptadecanes is characteristic of a direct contribution from

blue-green algae (e.g. Han, McCarthy and Calvin, 1968; Eglinton, Maxwell and Philp, 1974). Since steroidal alkanes (steranes) (e.g. Mulheirn and Ryback, 1975) are geological maturation products of sterols, their presence in contemporary environments is good evidence of a fossil fuel-derived input (Cardoso, 1976; Brassell and others, 1978).

The origins of certain other alkanes which are ubiquitous in recent sediments can only be determined from elucidation of their stereochemistry. Three examples have been chosen to illustrate this approach: 2,6,10,14-tetramethylpentadecane (pristane) (I), 17β(H)-hopane (II) and 17α(H)-homohopane (III, R=CH_3). Pristane occurs widely in marine organisms, and in zooplankton and a higher marine organism comprises solely the 6(R),10(S) isomer (Ia) (Cox and others, 1972; Patience, Rowland and Maxwell, 1979). In mature geological samples, including petroleum, the alkane comprises a mixture of Ia:Ib + Ic in the ratio 1:1 (Patience, Rowland and Maxwell, 1979). Thus, determination of the configuration of pristane in a recent sediment should allow distinction between a biological and a pollutant source.





Compound II also occurs widely in recent sediments (e.g. Brooks and others, 1977), but possible sources are more difficult to assign. It does not occur in petroleum, so is unlikely to derive from a pollutant source; there is only one report of its occurrence in an acidophilic bacterium (De Rosa and others, 1973).

Petroleum triterpanes are mainly of the 17α(H)-hopane type and are characterised ($\geq C_{31}$) by the presence of ca. 1:1 mixtures of C-22 diastereoisomers (e.g. Ensminger and others, 1974). The occurrence of such a distribution in recent sediments has been taken as evidence for petroleum-derived pollution (Dastillung and Albrecht, 1976). However, in many recent sediments the C_{31} member (III) occurs as an unequal mixture, with the later-eluting isomer (GLC) more abundant; this points to an additional (biological) origin for this isomer although it has never been found in organisms (Brooks and others, 1977). In the present study the origin of each of these compounds in a particular sediment has been determined by application of gas chromatography and computerised gas chromatography-mass spectrometry techniques: (i) pristane in a lacustrine sediment, (ii) 17β(H)-hopane in the same sediment, (iii) 17α(H)-homohopane in a sphagnum peat.

EXPERIMENTAL

Sample Collection

Rostherne sediment. Cores were collected using a Gilson mud sampler, and stored frozen before sectioning and analysis (Gaskell, 1974).

Microcystis aeruginosa. A sample of the alga was collected from the surface of the lake. An aliquot was kept for analyses. A second portion was suspended (18 wk) just below the lake surface (0.5m) in a conical flask with the neck packed with sterile glass wool; subsequently, it was allowed to stand (53 wk) on the lake bed (30m). The laboratory culture was grown by Mr.B. Capel at Porton Microbiological Research Establishment. Growth occurred (7d) at ambient in an aerated polycarbonate vessel (40ℓ) containing a mineral salts solution with aqueous garden soil extract. The cells were harvested by centrifugation.

Sphagnum cuspidatum. A fresh sample was collected from the Lyne of Skene peat bog. Part was kept for analysis whilst a second portion was allowed to decay (ca. 13 mnth) in a culture room at 30°C under aerobic conditions in the dark. In addition, a sample of the moss base was collected for analysis.

Extraction and Separation

Samples were extracted using either a Dawes soniprobe (sediment samples; i-PrOH/hexane, 4:1), a Soxhlet apparatus (algal samples; $(CH_3)_2CO$ followed by CH_2Cl_2/MeOH, 2:1), a direct reflux (moss base and decayed moss, CH_2Cl_2/hexane; living moss, $(CH_3)_2CO$ followed by CH_2Cl_2/MeOH, 2:1). In each case, the 'neutral' fraction of the total organic extract was separated from the 'acid' fraction by shaking with aqueous (or methanolic) KOH ($\leqslant$ 10% w/v; ca. 50ml) followed by extraction with hexane or CH_2Cl_2. The neutral fraction was separated by thin-layer chromatography (TLC) on SiO_2 (CH_2Cl_2 developer) to yield a hydrocarbon band (Rf $\simeq$ 0.8). Urea adduction (3X) separated the hydrocarbons into an adduct and non-adduct (branched and cyclic) components; the latter was further fractionated by TLC (10% $AgNO_3/SiO_2$; hexane developer) to yield the branched and cyclic alkanes (Rf $\simeq$ 0.7).

Gas Chromatography

Diastereoisomers of pristane were separated using a modified Perkin Elmer F-17 gas chromatograph (flame ionisation detector). This was fitted with a DEGS glass capillary column (100m) using helium (30 psig) as carrier gas. The oven temperature was programmed from 40°C to 80°C at 2°/min.

Gas Chromatography-Mass Spectrometry

All samples were analysed using a Finnigan 9610 gas chromatograph coupled directly to a Finnigan 4000 mass spectrometer. The chromatograph was fitted with an OV-1 glass capillary column (20m) using helium (ca. 10 psig) carrier gas. Temperature programme conditions were 50°C to 260°C at 6°/min. Typical mass spectrometer conditions were: ion source temperature 200°C, electron energy 70 eV, filament current 430 μA. Data were collected (ca. 1.5 sec per scan) on a DEC PDP 8/e (32K core) laboratory computer. Mass fragmentograms and mass spectra were plotted using a Calcomp 565 printer.

RESULTS AND DISCUSSION

Rostherne Mere, Cheshire (U.K.) is a small (0.5 km^2), eutrophic lake with permanent oxygen depletion at the deepest part (ca. 30m). The dominant algal species is the blue-green Microcystis aeruginosa, which is particularly abundant as intense blooms during the summer months. It is probably the major contributor of organic matter to the bottom sediment (Belcher and Storey, 1968; Reynolds and Rogers, 1976). Skene Moss (0.5 km^2) is situated near the Lyne of Skene, Aberdeenshire (U.K.) and is an oligotrophic, raised moss. The upper 2.5m of peat is composed mainly of Sphagnum cuspidatum remains; the peat has been used as a fuel so the present surface dates from the sub-Boreal period (2000-3000 yr B.P.) except for the upper 1cm deposited within the last 100 years (Wheatley, Greaves and Inkson, 1976).

All the hydrocarbons were identified from comparison of mass spectra with standards, except for those in the moss which were assigned by mass fragmentography (m/e 191, 205) and retention data.

Pristane in Rostherne Sediment

Pristane was analysed by GLC on diethyleneglycol succinate (DEGS). The diastereoisomer separation for three sediment sections (0-7cm, 7-18cm, 18-30cm), a sample of the alga collected from the lake surface, and a standard (1:1 mixture of the 6(R),10(R) and 6(R),10(S) isomers, Ib,a) is shown in Fig.1. The

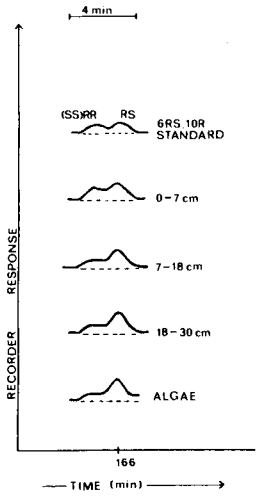


Fig. 1. GLC traces of pristane (100m DEGS): i) 1:1 mixture 6(R),10(R) and 6(R),10(S) isomers; ii) - iv) from different depths of Rostherne Mere sediment; v) from M.aeruginosa in Rostherne.

relative contribution from the 6(R),10(S) isomer (Ia) varied among the samples and was at a maximum in the alga. Since pristane of biological origin should comprise solely the 6(R),10(S) isomer (Cox and others, 1972; Patience, Rowland and Maxwell, 1979) each sample therefore contains a contribution from a pollutant source. A plot of the proportion of pollutant-derived pristane for each sample shows a relative decrease for this component with increasing depth of sediment, as would be expected (Fig. 2). The alga, although containing mainly the phytol-derived 6(R),10(S) isomer (Ia), still has a significant contribution from a contamination origin. The most likely source of the pollution is petroleum-derived hydrocarbon material entering the lake via a small sewage

effluent and/or run-off from a nearby trunk road.

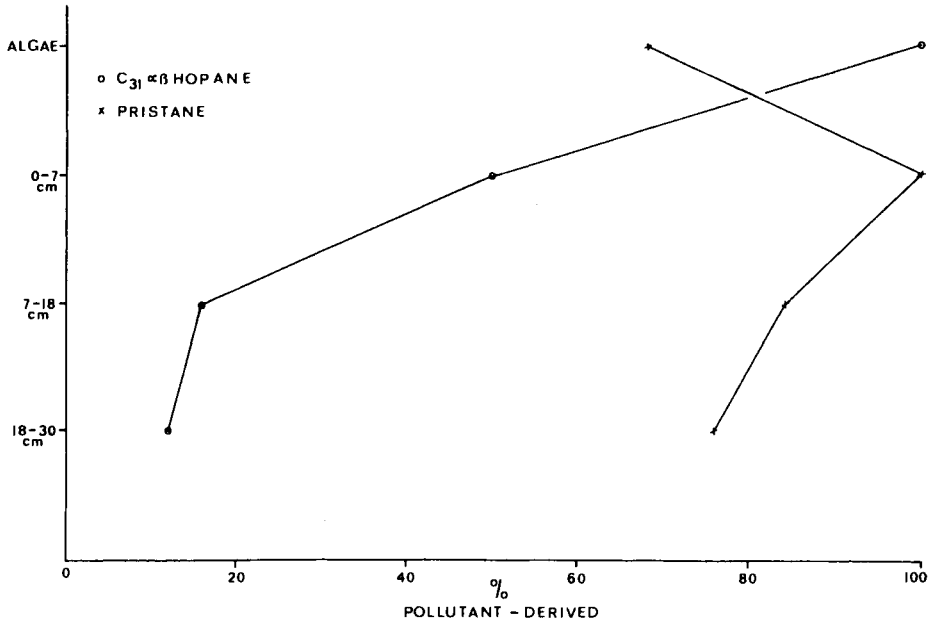


Fig. 2. Plot of percent contribution of pollutant-derived pristane and 17α(H)-homohopane in Rostherne alga and different sediment depths.

17β(H)-Hopane in Rostherne Sediment

Pentacyclic triterpanes of the hopane type show an abundant ion at m/e 191 in their mass spectra and can be recognised readily in complex mixtures by mass fragmentography, using this ion. Fig. 3D shows the m/e 191 fragmentogram of the alkane fraction from a sediment core (0-20cm). The extended (> C_{31}) 17α(H)-hopanes (III, R=C_2H_5,n-C_3H_7,n-C_4H_9,n-C_5H_{11}) occur as ca. 1:1 doublets for the two C-22 diastereoisomers (III). The distribution is that typically observed in mature geological samples and indicates that these components arise from a petroleum-derived source (Dastillung and Albrecht, 1976). The C_{30} alkane, 17β(H)-hopane is also a significant component (Fig. 3D) but cannot derive from a pollutant source (see above). Formally, it can only arise from a contemporary biological source or from an erosion of an immature ancient sediment where the 17β(H)-hopanes have not been replaced by the more stable 17α(H) series typical of mature samples (e.g. Ensminger and others, 1974; Van Dorsselaer, Albrecht and Ourisson, 1977). In Rostherne Mere, the geology of the area is such that the latter possibility is not likely and a biological origin is more feasible. Mass fragmentography reveals, however, the absence of 17β(H)-hopane in the cultured sample of M.aeruginosa (Fig. 3A). The two most abundant hopane derivatives recognised (2 and 390 ppm dry weight respectively) were hop-13(18)-ene (IV; Fig. 3A) in the alkane fraction (hindered double bond) and hop-22(29)-ene (V) in the alkenes. In the sample of the alga from the lake surface and the sample suspended in the lake prior to lowering

to the lake bed, the concentrations (ppm dry weight) were: hop-13(18)-ene, 5 and 6 respectively (Fig. 3B,C); hop-22(29)-ene, 820 and 450; 17β(H)-hopane, 0.3 and 2 (Fig. 3B,C). This is strong circumstantial evidence that the hopane originates from reduction of the abundant hop-22(29)-ene present. The culture sample, although not axenic, had no detectable iso- or anteiso-acids (C_{15} and C_{17}) of bacterial origin, whereas the lake and lake bed samples had both iso- and anteiso-acids present (70 and 1290 ppm total, respectively) (Quirk and Maxwell, unpublished results). It is possible, therefore, that the hopane arises from bacterial reduction of the alkene. This may represent a general pathway for the origin of the widespread 17β(H)-hopane in recent sediments.

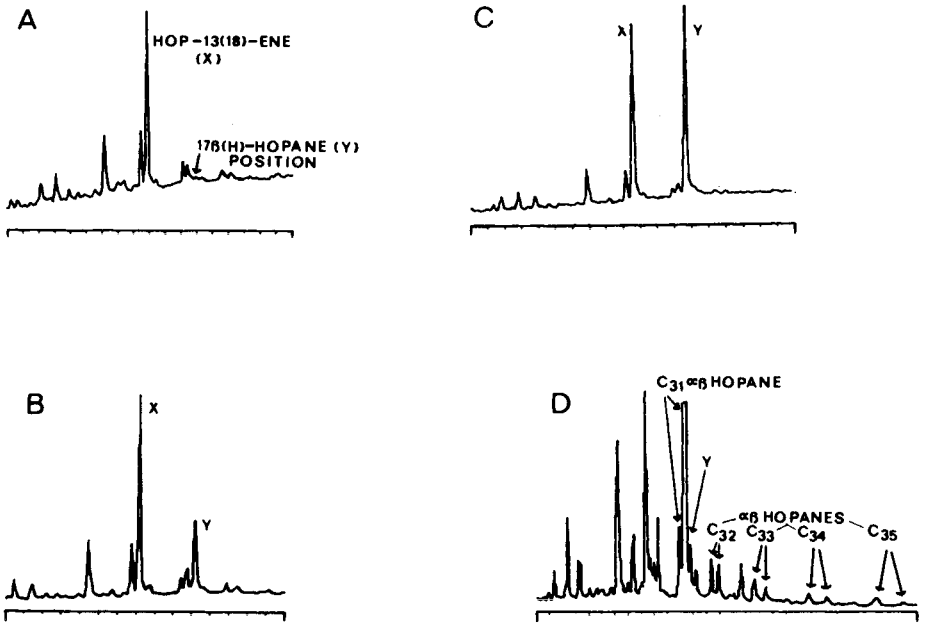


Fig. 3. Mass fragmentograms (m/e 191) of branched/cyclic alkanes from: A. Culture of M. aeruginosa; B. Rostherne surface alga; C. Rostherne alga (lake bed experiment); D. Rostherne sediment (0-20cm).

Of the two C-22 diastereoisomers of 17α(H)-homohopane (III, R=CH_3) in the sediment (Fig. 3D), the second isomer (by GLC) is in much higher relative abundance, unlike the C_{32} - C_{35} members of pollutant origin alone. The second isomer is, therefore, mainly of biological origin, and the pollutant contribution (both isomers in ca. 1:1 ratio) decreases (Fig. 2) with increasing depth of sediment (cf. pristane).

17α(H)-Homohopane in Lyne of Skene Peat

More detailed information about the origin of the second eluting isomer in the sedimentary environment was obtained by examination of the pentacyclic triterpanes in this peat and in a major contributor of organic matter, Sphagnum

cuspidatum. GC-MS analysis of the alkanes from various depths of a peat core showed the major triterpane by far to be 17α(H)-homohopane (III, R=CH_3) (Quirk and Maxwell, unpublished results). A similar situation has been observed in another peat (Gaskell, 1974) and in a lignite (Van Dorsselaer, Albrecht and Connan, 1977) and it is possible that this remarkable predominance in the branched and cyclic alkanes may be characteristic of certain peat types and their ancient counterparts. The branched and cyclic alkanes of the living moss showed only a trace amount of 17α(H)-homohopane, which was only revealed by mass fragmentography of m/e 191 (Fig. 4A) and 205 (not shown). The alkane was present as a ca. 1:1 ratio of the two C-22 isomers, indicating a pollutant origin. In the moss base (collected immediately below the living moss, age ca. 1 yr) a dramatic increase in the relative abundance of the second eluting isomer was observed (Fig. 4B). This situation was paralleled in the branched and cyclic alkane fraction from the moss sample allowed to decay under dark, aerobic conditions (Fig. 4C). The origin of the abundant second isomer of 17α(H)-homohopane (III, R=CH_3) in the peat, therefore, appears to be associated with bacterial decay. It is not known if it arises from alteration of a precursor in the moss or if it is solely a bacterial product. The latter appears more likely since extended (> C_{30}) hopane derivatives have only been found in bacteria and blue-green algae (e.g. Rohmer and Ourisson, 1976).

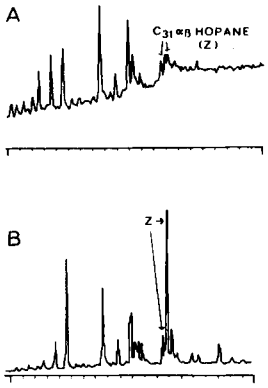
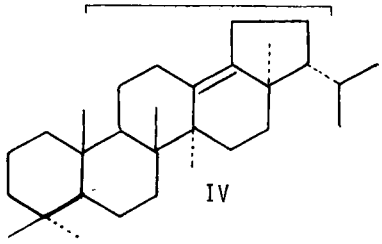
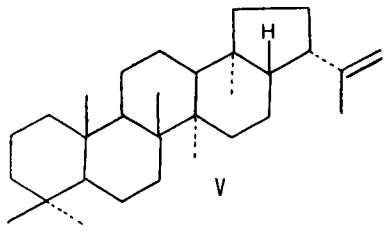


Fig. 4. Mass fragmentograms (m/e 191) of branched/cyclic alkanes from S. cuspidatum: A. Living moss; B. Moss base; C. Decayed moss (laboratory).





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Patterns of Hydrocarbon Contamination in California Coastal Waters

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ABSTRACT

First year results of the State of California's Mussel Watch Program indicate that the concentrations of complex hydrocarbon mixtures accumulated from seawater in the intertidal zone by mussels, Mytilus californianus, are closely related to human population density in adjacent coastal regions. High concentrations of the unresolved component were also observed in mussels obtained in the vicinity of a natural petroleum seep. These mixtures most likely consist in part of petroleum and petroleum-derived compounds. Mass chromatographic analysis of mussel and large-volume seawater extracts for the parent ions of hopanes, steranes, and monoaromatic steranes, - compounds present in characteristic ratios and abundances in crude oils, - appears to provide a method for distinguishing unresolved mixtures of petroleum origin from those which derive from other sources, as well as for determining possible origins of the petroleum component. A comparison of the levels of the unresolved component in the saturate fraction and of several individual hydrocarbon compounds in extracts of mussels from three diverse areas of southern California with corresponding levels in both the "dissolved" and particulate fractions of seawater sampled in the vicinity of the mussel beds indicates a variation over two orders of magnitude in the value of the partition coefficients. Although the factors responsible for this variation in uptake and/or accumulation are presently imperfectly known, mussels appear nevertheless to be a satisfactory first order biological indicator of the impact of man on the hydrocarbon content of coastal waters.

Key Words: Coastal pollution, petroleum pollution, chlorinated hydrocarbons, mussels, in situ seawater sampling, steranes, mass-fragmentography.

INTRODUCTION

This paper provides a preliminary and partial answer to the question "By how much has the chemistry of the coastal waters of California changed as a result of the activities of man?" Answers to this question and to the companion

question "What is the direction of change?" would clearly assist in the formulation of long term policies of management and protection. The California coastal zone receives the wastes of many millions of people, is the site of expanding economic activities, including tanker traffic of crude oil and refined petroleum products, possesses submarine petroleum reserves that are now being exploited, but also contains many areas of incomparable natural beauty and biological diversity. It has long been known that the waters of the California coastal zone are contaminated by organochlorine compounds of the DDT and PCB groups (Risebrough and colleagues, 1976); this paper examines the present distribution of petroleum and petroleum-related hydrocarbons, for which relatively few data have thus far been obtained.

Because methodologies for the sampling and measurement of organic pollutants, including petroleum compounds, in seawater are still in a state of rapid development (de Lappe and colleagues, 1979a), recent monitoring programs of coastal water quality have relied heavily on biological indicators of local pollution levels, in particular mussels, Mytilus sp., and other bivalves. Data presented in this paper were obtained in two such California programs: 1) the California State Mussel Watch Program, which is examining levels of pollutants accumulated by the mussel M. californianus from seawater in the intertidal zone of a series of biological reserves along the California coast designated as "Areas of Special Biological Significance"; 2) a three year baseline program undertaken by the U.S. Bureau of Land Management prior to the initiation of offshore drilling for petroleum in southern California. Two supplemental questions relevant to the interpretation and significance of the data are also addressed: 1) although it is well established that mussels accumulate a variety of pollutants from the ambient seawater system (Goldberg and colleagues, 1978), how are the pollutant levels in the mussels related to those in both the "dissolved" and particulate phases of seawater? 2) Gas chromatographic profiles of weathered petroleum from almost any source invariably contain a "hump" consisting of an unresolved complex mixture (UCM) (Farrington and Meyers, 1975). To varying degrees, the UCM is also present in chromatograms of extracts of mussels from both areas of wastewater discharge as well as areas which are relatively pristine. How can the UCM's containing petroleum compounds be distinguished from those consisting of, or containing, complex mixtures of other hydrocarbons and related compounds, both anthropogenic and biogenic? Also, how can the UCM's of petroleum from different sources be distinguished? This is clearly a desirable goal in the study of coastal pollution patterns.

EXPERIMENTAL

Mussels were obtained from thirty of the Areas of Special Biological Significance in July, 1977, when upwelling generally occurs along the California coast, and again in November. Collection sites were: 1) San Miguel Island, west; 2) San Miguel Island, east; 3) Santa Cruz Island; 4) Anacapa Island; 5) Santa Barbara Island; 6) Santa Catalina Island; 7) La Jolla; 8) Oceanside; 9) Corona del Mar; 10) Royal Palms; 11) Point Mugu; 12) Goleta Point, reference station; 13) Pt. Conception; 14) Pt. Arguello; 15) Montana del Oro; 16) Salmon Creek; 17) J.P.Burns State Park; 18) Soberanes Pt.; 19) Carmel; 20) Pt. Pinos; 21) Ano Nuevo Island; 22) Fitzgerald; 23) Farallon Islands; 24) Berkeley Pier, a reference station (M. edulis); 25) Point Reyes; 26) Bodega Head; 27) Salt Point; 28) Pygmy Forest; 29) Shelter Cove; 30) Humboldt Bay; 31) Trinidad Head; and 32) Klamath River. Collection sites in southern California are shown in Figure 1.

Analytical methods have been described elsewhere; approximately 40 mussels

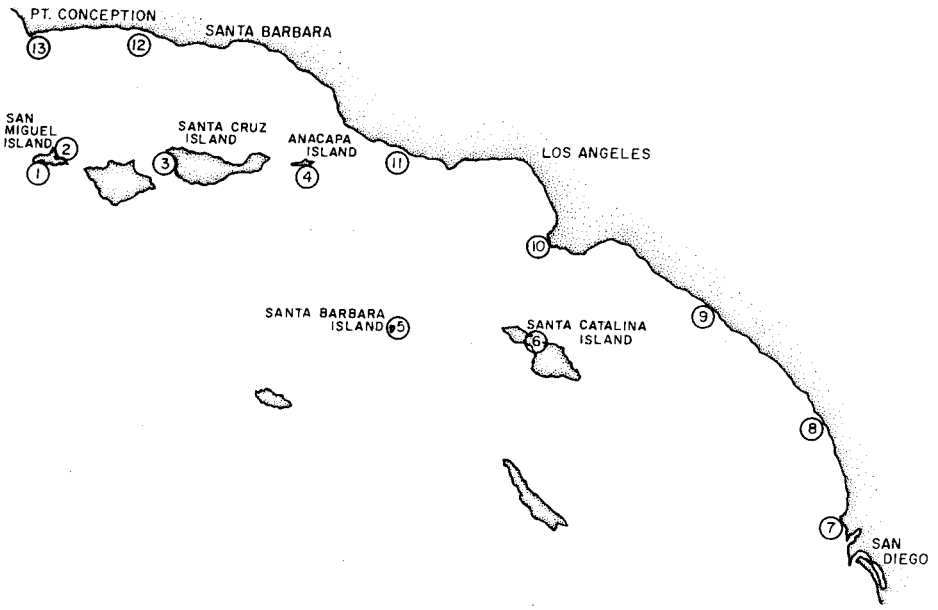

Figure 1. Collection sites of the California State Mussel Watch Program in the Southern California Bight in 1977.

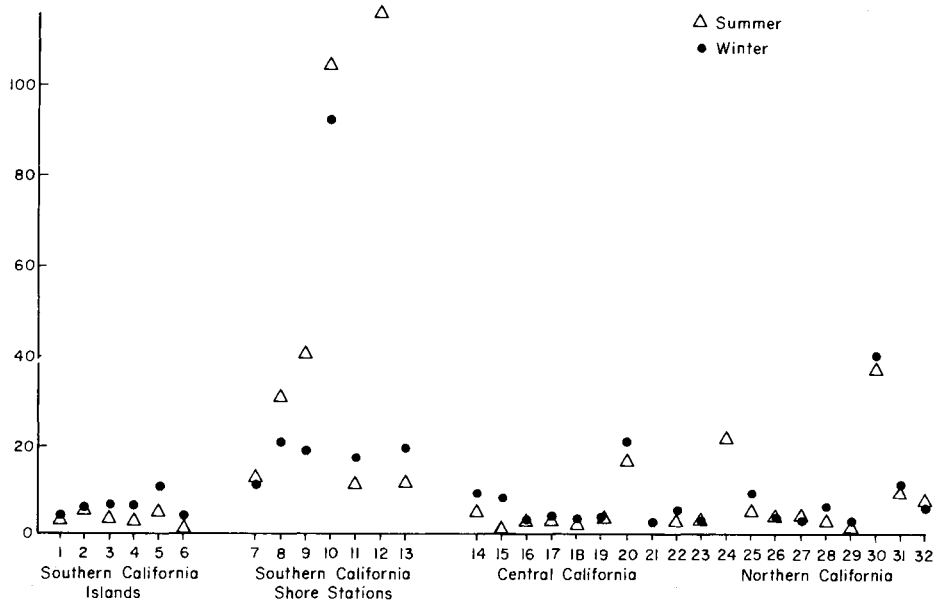

Figure 2. Distribution of concentrations of the unresolved component in saturate fractions of Mytilus along the California coast. Concentrations in micrograms/gram of the dry weight. Station sites are listed in the text.

from each site were pooled to form a composite sample to reduce sampling variance (Risebrough and colleagues, 1978). Seawater samples were obtained in situ from R. V. INVINCIBLE at Goleta Point (34°24.1'N, 119°50.5'W), 1260 and 1474 liters on 4-5 Sept., 1977; at Dutch Harbor, San Nicholas Island (33°13.0'N, 119°29.0'W), 1275 and 1333 l, on 10-11 Sept., 1977; and on a transect between Pt. Fermin and Pt. Vicente (33°43.3'N, 118°25.0'W to 33°40.5'N, 118°17.5'W), 1456 and 1314 l, on 15-16 Sept., 1977. Water was collected through a stainless steel and teflon tube mounted off the bow of the vessel with a Jupiter teflon impeller pump and passed through a precombusted glass fiber filter (293 mm, 0.3 μm) and two teflon columns packed with high density polyurethane foam, mounted in parallel. Flow rates and sample volumes were monitored by turbine flowmeters. Additional sampling details and analytical methodologies are described elsewhere (de Lappe and colleagues, 1979b).

RESULTS AND DISCUSSION

The distribution of the concentrations of the unresolved component (UCM's) in the saturate fraction in mussels along the California coast is shown in Figure 2. Levels at the shore stations of the Southern California Bight (7-13) are elevated over those on the offshore islands (1-6; Figure 1). Station 12, Goleta Point, is the site of a natural oil seep. Levels were low in sparsely inhabited areas and significantly higher in the vicinity of Monterey (20), in San Francisco Bay(24), and in Humboldt Bay(30). With the exception of Goleta Point, the distribution of the unresolved component parallels closely the human population density along the coastal zone. We explain this distribution by attributing it to the discharge in wastewaters of petroleum, refined petroleum products, other hydrocarbons and various synthetic chemicals.

Using mass chromatography, Seifert and Moldowan (1978) have been able to distinguish a number of components of the UCM of California crude oils, including hopanes, steranes and monoaromatic steranes. These compounds are present in crude oils in characteristic ratios and abundances. This method of fingerprinting provides not only a technique for distinguishing among crude oils, but also for differentiating between petroleum-derived mixtures and complex mixtures from other sources. It has been used to distinguish among tar balls of different origins in the Mediterranean (Albaigés and Albrecht, 1979). Multiple ion monitoring, using a Finnigan 4023 quadripole mass spectrometer, equipped with a 30 m, 0.25mm I.D., SP-2100 (J&W Scientific) glass capillary column, of mussel extracts for characteristic ions of hopanes (m/e 191), steranes (m/e 217) and dimethyl monoaromatic steranes (m/e 253) revealed the presence of series of peaks within the UCM for these ions. Mass fragmentograms of m/e 253 of the mussel extracts from Goleta Point and Palos Verdes (Royal Palms, near a large wastewater outfall) and of the seawater particulate fraction from Goleta Point are shown in Figure 3. These preliminary results indicate that the technique can be used to obtain qualitative differences among unresolved mixtures which can not be distinguished by gas chromatographic techniques alone; they also provide a direction for continuing research.

The development of large-volume in situ seawater sampling techniques (de Lappe and colleagues, 1979a, 1979b) has permitted a comparison of hydrocarbon levels in mussels with those in the "dissolved" and particulate fractions of seawater sampled in the vicinity of the mussel beds. Flame ionization glass capillary chromatograms of extracts of the dissolved and particulate phases of seawater obtained on the transect off the Palos Verdes Peninsula and of mussels at Royal Palms are shown in Figure 4. Concentrations of the total unresolved component in the saturate fraction and of DDE, PCB, pristane and squalene

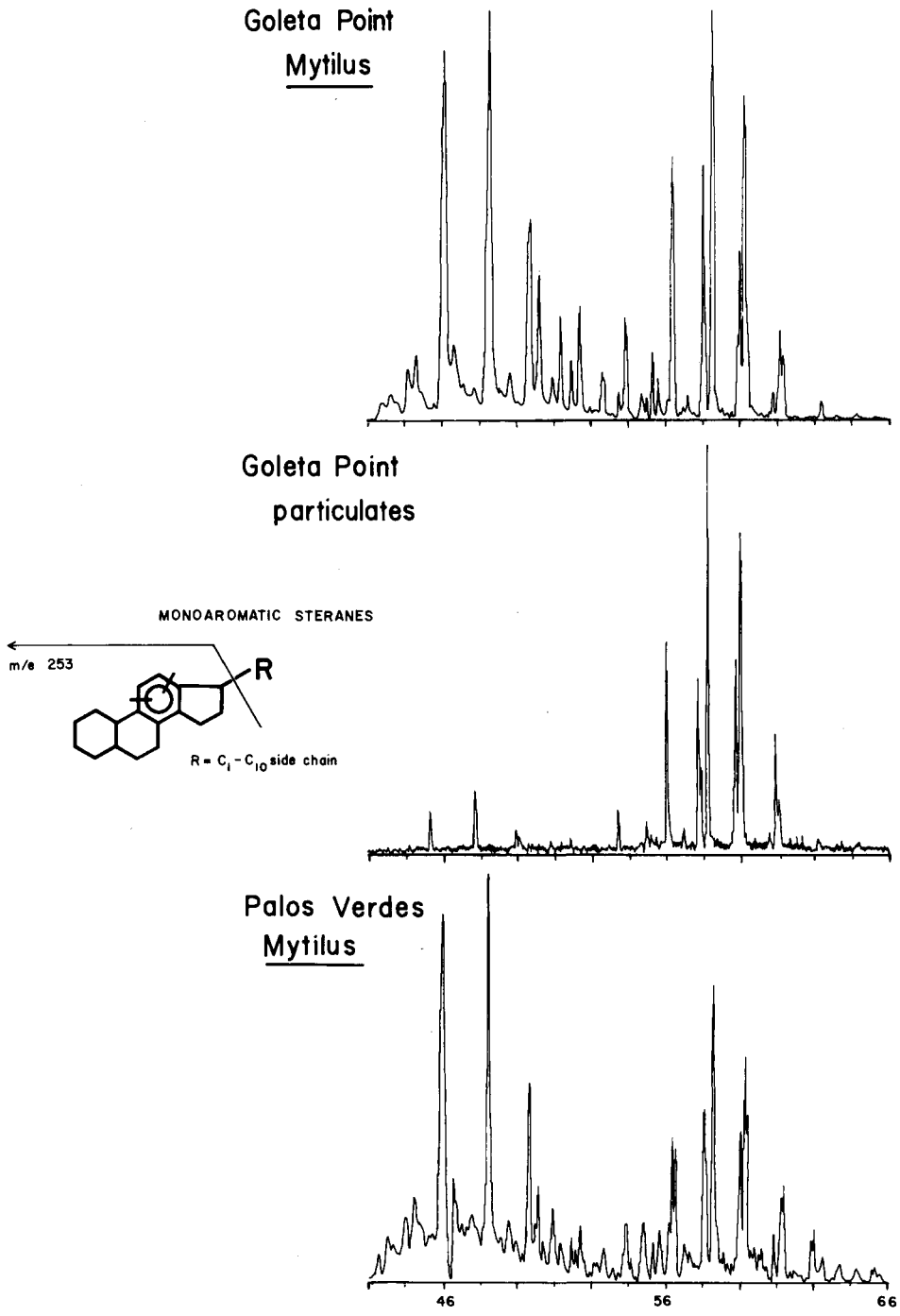

Figure 3. Mass chromatograms, m/e 253 ± 0.5, characteristic of dimethyl monoaromatic steranes, of extracts of Mytilus from Goleta Point and Palos Verdes Peninsula, and of seawater particulates from Goleta Point. Time in minutes.

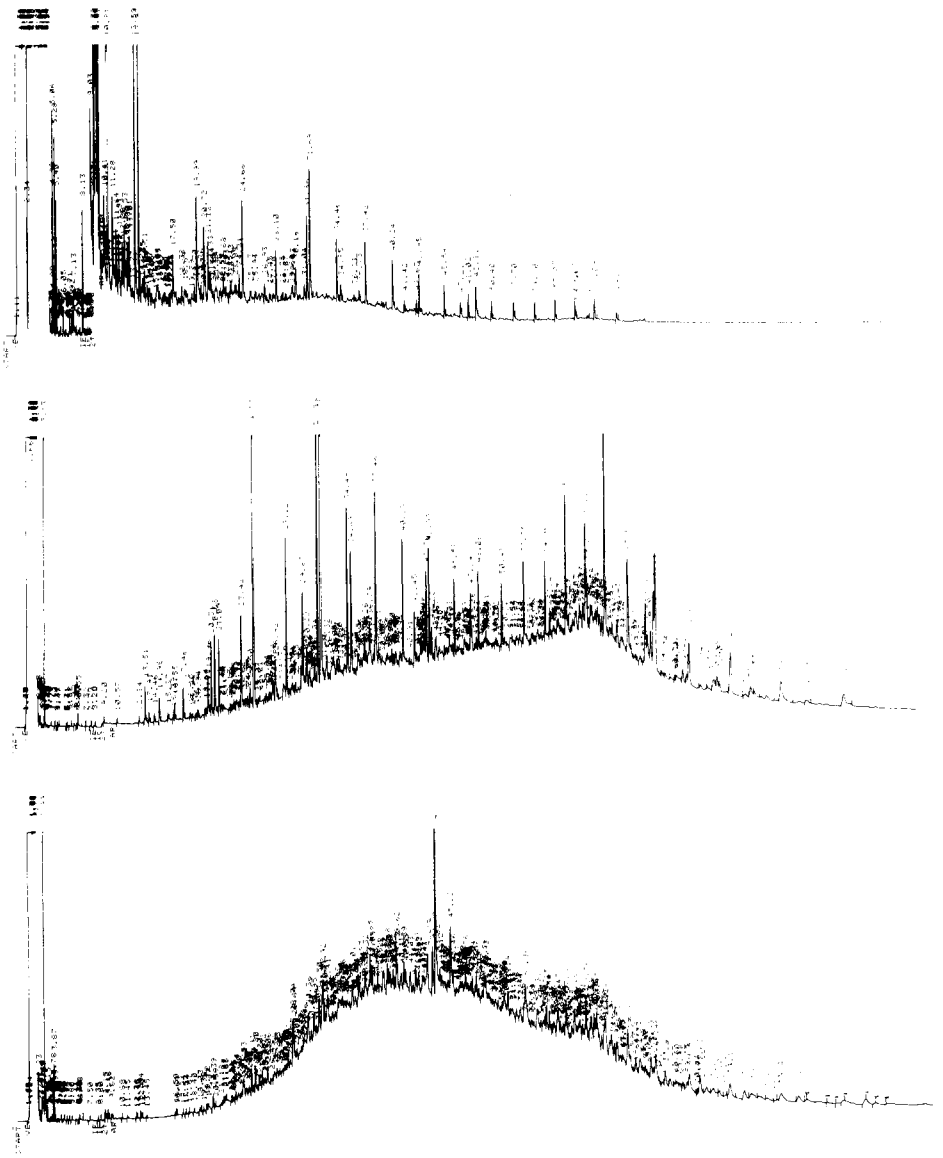

Figure 4. Glass capillary flame ionization chromatograms of saturate fractions from "dissolved" (top) and particulate (middle) phases of composite seawater samples and Mytilus californianus (bottom) from the Palos Verdes Peninsula of Southern California. Chromatographic conditions: Hewlett-Packard 5840A gas chromatograph; 65°-270°, rate 3.5°/min.; 30 meter column, SP-2100, 0.25 mm I.D. (J&W Scientific): 2.0 µl injections, splitless mode.

Table 1. Partitioning of hydrocarbons among the mussel, Mytilus californianus, and "dissolved" and particulate phases of seawater. Concentrations in "dissolved" (N=2, with standard deviation) and particulate phases in ng/liter, concentrations in mussels in ng/g. Southern California Bight, Aug.-Sept., 1978. Partition coefficients (ratio of ng/kg in mussels to ng/liter in seawater) are given in parentheses.

		Palos Verdes		Goleta Point		San Nicholas Island	
DDE	"dissolved" phase	0.18±.02	(6×10^6)	0.073±.024	(3×10^5)	0.044±.007	(4×10^5)
	particulates	.082	(1×10^7)	.072	(3×10^5)	.008	(2×10^6)
	mussels	1100		22		18	
PCB	"dissolved" phase	0.13±.03	(3×10^6)	0.074±.011	(4×10^5)	0.10±.04	(6×10^4)
	particulates	.044	(1×10^7)	.019	(1×10^6)	.011	(5×10^5)
	mussels	440		27		6	
Pristane	"dissolved" phase	0.061±.03	(7×10^5)	0.036±.011	(2×10^7)	0.20±.00	(6×10^5)
	particulates	2.0	(2×10^4)	5.2	(1×10^5)	2.2	(6×10^4)
	mussels	40		640		120	
Squalene	"dissolved" phase	0.74±.35	(7×10^6)	0.25±.07	(7×10^6)	0.35±.16	(4×10^6)
	particulates	32	(2×10^5)	4.9	(4×10^5)	30	(4×10^4)
	mussels	4900		1700		1300	
Total unresolved saturates	"dissolved" phase	4.1±1.3	(3×10^7)	5.6±1.2	(7×10^7)	12±2	(3×10^5)
	particulates	83	(2×10^6)	970	(4×10^5)	13	(3×10^5)
	mussels	140,000		390,000		3,900	

of these and other samples are presented in Table 1. The ratio of the resolved to the unresolved component in the mussels (.06) is substantially lower than in either the particulates (.17) or the "dissolved" component (0.71). Differences of equivalent magnitude were also observed among the chromatograms of the aromatic fractions of seawater and mussels from Palos Verdes, and in extracts from Goleta Point and San Nicholas Island. Among the partition coefficients (ratio of concentrations in mussels to those in water) presented in Table 1, there is a variation over two orders of magnitude. Reasons for this variation are presently unknown, but may include a high variance in the water sampling, - samples of seawater were obtained, however, over a full tidal cycle of 25 hours to minimize this component of variance - differences in the microenvironment of the surfzone and offshore, levels of dissolved organic material, etc. In spite of the observed variation, calling for additional studies to permit fuller interpretation of the data, the results presented in Figure 2 indicate that mussels appear to provide a meaningful first order estimate of the magnitude of chemical change in the California coastal environment.

ACKNOWLEDGEMENTS

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Determination of Trace Level Hydrocarbons in Marine Biota

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ABSTRACT

A method is described for the determination of hydrocarbons in marine biota. This method utilizes dynamic headspace sampling of an aqueous caustic tissue homogenate to extract and collect volatile organic components. Interfering polar biogenic (non-anthropogenic) components are removed by normal-phase high-performance liquid chromatography (HPLC) prior to quantitation and identification of the hydrocarbons by gas chromatography and gas chromatography-mass spectrometry. After headspace sampling the non-volatile polycyclic aromatic hydrocarbons are solvent extracted from the tissue homogenate, isolated using normal-phase HPLC, and analyzed by reversed-phase HPLC with ultraviolet (UV) and fluorescence detection.

Results of an interlaboratory comparison of determinations of hydrocarbons in mussel tissue are also reported.

KEY WORDS: aliphatic hydrocarbons, aromatic hydrocarbons, gas chromatography, headspace sampling, high-performance liquid chromatography, interlaboratory comparison, polycyclic aromatic hydrocarbons.

INTRODUCTION

The low concentration of hydrocarbons encountered in marine petroleum pollution monitoring and baseline studies necessitates the development of analytical techniques for biota which are sensitive at the µg/kg level. Since most of the organic compounds present are of biological origin, a suitable chemical cleanup of the sample is necessary in order to remove interfering non-hydrocarbon compounds prior to the measurement of the low levels of hydrocarbons present. Furthermore, analytical methods which ultimately permit the identification of individual hydrocarbon components are desired.

A rapid and sensitive method for the determination of hydrocarbons in marine biota has been developed in this laboratory. This method utilizes dynamic headspace sampling of an aqueous caustic tissue homogenate to extract and collect the volatile organic components. Interfering polar biogenic (non-anthropogenic) components are removed by high-performance liquid chromatography (HPLC) prior to quantitation and identification of the hydrocarbons by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). After headspace sampling the non-volatile hydrocarbon components (e.g., polynuclear aromatic hydrocarbons (PAHs)) are solvent extracted from the caustic tissue homogenate, isolated using normal-phase HPLC, and analyzed by reversed-phase HPLC with ultraviolet (UV) and fluorescence detection.

In contrast to previously reported methods for tissue, this headspace sampling procedure is applicable to the determination of low levels of hydrocarbons in relatively pristine areas as well as greater levels found in organisms exposed to petroleum. The application of this method for the determination of hydrocarbons in mussels, clams, and oysters will be discussed.

Many laboratories, using a variety of different analytical techniques (Medeiros and Farrington, 1974; MacLeod and coworkers, 1976; Warner, 1976; Shaw and Baker, 1978), are currently involved in the measurement of hydrocarbons in marine biota. At present, there is little or no knowledge of the comparability of data from different laboratories. In order for the data from diverse methods to be useful and reliable, there must be a basis for intercomparability. Furthermore, unless these data can be put on an equivalent basis, environmental standards can neither be rationally set nor fairly enforced. Intercalibration studies on the determination of hydrocarbons in spiked tuna meal samples (Farrington and coauthors, 1976) and marine sediments (Hilpert and coworkers, 1978) have been reported. Such intercomparison studies are necessary to determine the environmental significance of analytical determinations generated by different laboratories using different methods. Preliminary results of an eight laboratory intercomparison exercise for the measurement of hydrocarbons in two mussel (*Mytilus*) samples are described in this paper.

EXPERIMENTAL

Headspace Sampling Method

The headspace sampling procedure developed in this laboratory for the determination of hydrocarbons in marine tissue has been described in detail previously (Chesler and coworkers, 1978). A schematic diagram of this procedure is shown in Fig. 1.

Interlaboratory Exercise

The *Mytilus* samples used in the intercomparison exercise were collected from two sites: Simpson Bay in the Northeastern Gulf of Alaska, a relatively pristine area, and Coal Oil Point, adjacent to natural oil seeps near Santa Barbara, California. The *Mytilus* were frozen in dry ice immediately after collection and remained frozen until homogenization. Approximately 2 kg of tissue from each site were homogenized for 1.5 hours using a Brinkman ultrasonic homogenizer. Aliquots of the homogenate ($\sim$40 g) were transferred to

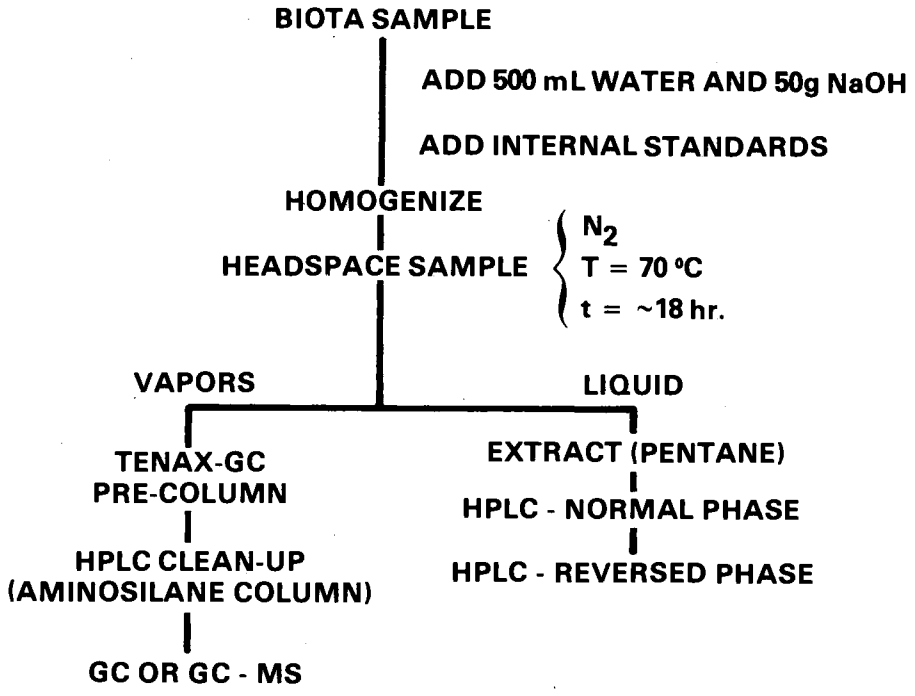

Fig. 1. Schematic diagram for the determination of hydrocarbons in marine biota.

50 mL glass bottles, refrozen, and stored at -10 °C. Each participating laboratory received two bottles from each site for analysis.

For the purposes of this interlaboratory comparison the following data were requested:

1. Total hydrocarbons in the GC elution range (approximately nC_{10}-nC_{30}).
2. Total extractable hydrocarbons.
3. Pristane/phytane ratio and the amount of each present.
4. Percent water.
5. Identities and amounts of the three most abundant aliphatic and three most abundant aromatic hydrocarbons in the GC range.
6. Total polynuclear aromatic hydrocarbon (PAH) concentration (four rings and larger).
7. Identity and amount of the most abundant PAH (four rings and larger).
8. All additional single compound identifications and concentrations made in the laboratory.

All concentrations were reported on a dry weight basis.

The analytical methods employed by the other laboratories participating in the intercomparison exercise consisted of: (1) aqueous or alcoholic digestion of the tissue, (2) extraction with an organic solvent (i.e., hexane or diethyl-ether), (3) isolation of the saturated and unsaturated hydrocarbons using silica gel column chromatography, (4) separation and quantitation of the hydrocarbons by capillary GC, and (5) identifications by GC retention times and/or GC/MS. Each laboratory used slight variations of this general approach.

RESULTS AND DISCUSSION

Headspace Sampling Method for Hydrocarbon Determination

Preliminary GC/MS investigations of headspace sampled *Mytilus* homogenate indicated that the major biogenic compounds present on the TENAX-GC trap after headspace sampling were long chain aliphatic alcohols. Since these biogenic compounds interfere with the GC analysis of the petroleum hydrocarbons, an HPLC cleanup procedure was employed to separate the hydrocarbons from the more polar biogenic compounds. Normal-phase HPLC, using an aminosilane bonded phase column, was used to achieve the isolation of the hydrocarbons. The long chain aliphatic alcohols are retained on the column and they can be removed by increasing the polarity of the mobile phase.

The effective removal of the more polar biogenic components by this HPLC technique is demonstrated in Table 1. These data from various tissue samples (mussels, oysters, and clams), and a sediment sample indicate that HPLC

TABLE 1 Comparison of Volatile Hydrocarbon Levels Obtained with and without HPLC Cleanup, µg/kg

	No HPLC		HPLC	
Mytilus (mussels)	2514 ± 76[a]	(2)[b]	1276 ± 179	(3)
(Northeastern Gulf of Alaska)	1406 ± 98	(2)	540 ± 46	(3)
Oysters (Middle Marsh, S.C.)	1834	(1)	652	(1)
Clams A (control-no crude oil exposure)	509 ± 11	(2)	377 ± 88	(2)
Clams B (1 µg crude oil/g water)[c]	1421 ± 114	(2)	491 ± 88	(3)
Clams C (10 µg crude oil/g water)[d]	1704	(1)	1413 ± 398	(2)
Sediment (collected near natural oil seep)	566 ± 37	(2)	574	(1)

[a]Data reported as the standard deviation (1 σ) of a set of replicate values from the mean of the replicate values.

[b]Denotes the number of samples analyzed.

[c]Exposed to 1 µg crude oil/g of water.

[d]Exposed to 10 µg crude oil/g of water.

removal of the non-hydrocarbon components is necessary to determine low hydrocarbon levels in tissue effectively. Of particular interest in Table 1 are the results obtained with various clam samples with and without exposure to 1 and 10 µg of crude oil/g of water. A comparison of the data for the control clams with and without HPLC cleanup (Fig. 2A and 2B, respectively) reveals that the six most abundant components (~100 µg/kg total) in the sample without HPLC cleanup are non-hydrocarbon. These six peaks are labeled *a* through *f* in Fig. 2B. A comparison of the results obtained after HPLC and after excluding the control level (i.e., 400 µg/kg), for the clams exposed to 1 µg and 10 µg crude oil/g of water shows a difference of a factor of 10 in petroleum uptake. The data in Table 1 support the applicability of the above method for the determination of hydrocarbons in marine organisms exposed to toxic levels as well as those from unpolluted environments.

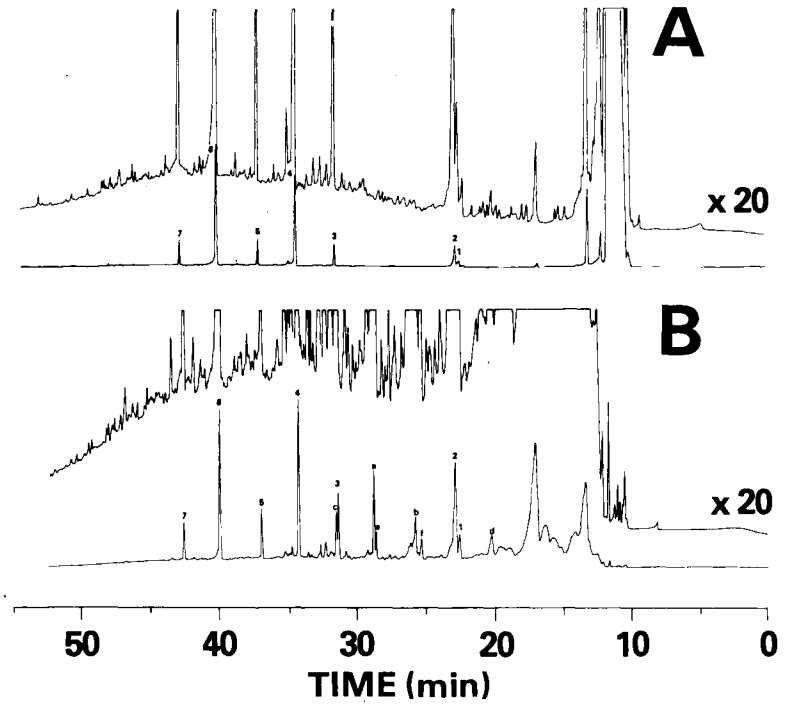

Fig. 2. Dual trace (two different attenutations) gas chromatograms of headspace sampled clams (A) with HPLC cleanup and (B) without HPLC cleanup. Column and Conditions: glass SE-30 coated SCOT column (100 m X 0.75 mm i.d.). Conditions: Helium at 6 mL/min, 4 min isothermal at 80 °C, then 4 °C/min to 275 °C and hold, flame ionization detection. Internal standards added for quantitation: (1) 2-methylundecane, (2) naphthalene, (3) 5-methyltetradecane, (4) 2,3,6-trimethylnaphthalene, (5) 7-methylhexadecane, (6) phenanthrene, and (7) 2-methyloctadecane. A detailed discussion of the method of quantitation and recovery data using these internal standards has been described (Chesler and coworkers, 1976, 1978). Peaks *a* through *f*: non-hydrocarbon compounds removed by HPLC cleanup.

To evaluate the effect of the HPLC cleanup on petroleum hydrocarbons, a sediment sample with a natural low-level petroleum hydrocarbon burden and with essentially no biogenic contamination was analyzed with and without HPLC cleanup. The data in Table 1 for this sediment indicate that the measured petroleum hydrocarbon level determined by the headspace sampling procedure is, as expected, unaffected by the HPLC cleanup.

After removal of the volatile hydrocarbons by headspace sampling, the nonvolatile PAHs are determined by solvent extraction and HPLC/fluorescence analysis. Individual PAHs can be identified by using normal-phase HPLC to isolate the PAHs according to the number of condensed aromatic rings, and then subsequent analysis of the fractions by reversed-phase HPLC with fluorescence detection as described previously. (Wise and coworkers, 1977).

The headspace sampling procedure for the analysis of hydrocarbons in marine biota offers several advantages over current solvent extraction procedures. When compared to solvent extraction methods, the headspace sampling technique requires minimal sample handling, few sample transfers, and only a minimal amount of organic solvent, thereby reducing the risk of contamination (a system blank for the headspace sampling method results in a value of only $\sim$5 µg/kg based on a sample of 600 mL of water). In addition, only one solvent concentration step is involved (i.e., after the HPLC cleanup), thereby reducing the losses of the more volatile components. When compared to solvent extraction procedures, the analyst's time is greatly reduced using the headspace sampling technique.

Interlaboratory Exercise

This intercomparison exercise was conducted to determine the adequacy of analytical procedures for hydrocarbon determinations in mussel tissue and to indicate the reliability with which results from different laboratories may be compared. Homogeneity and stability studies were conducted at NBS using the dynamic headspace sampling technique to measure total hydrocarbons in the GC elution range. The results of these studies are summarized in Table 2. Both the Santa Barbara and Alaskan mussel homogenates showed a nearly two-fold increase in total hydrocarbons after two months of storage. Data from a previous storage stability study on a similar tissue homogenate also revealed an increase of approximately two times the total hydrocarbon value after a 40 day study. The reasons for this increase are currently under investigation. The percent relative standard deviations for the analyses of Alaskan and Santa Barbara mussels after the initial two months of storage were 14 percent and 18 percent, respectively.

The results of the percent water analyses for all laboratories were 88.6 ± 1.7 percent for the Alaskan Sample and 85.3 ± 2.5 percent for the Santa Barbara sample. The interlaboratory comparison results for total hydrocarbons in the GC elution range, total extractable hydrocarbons, and pristane/phytane ratio are given in Tables 3 and 4. The results for the total hydrocarbons in the GC elution range have been divided into subtotals for aliphatic and unsaturated/aromatic hydrocarbons. The quantitation of the peaks in the gas chromatograms included subtotals for the resolved peaks and the unresolved complex mixture (UCM). A comparison of the total hydrocarbons data indicate that the Santa Barbara mussel homogenate has a greater concentration of hydrocarbons than the Alaskan mussel homogenate. However, the ranges for the amounts of the resolved peaks for the aliphatic hydrocarbons in both samples

are similar. The pristane/phytane ratios for the two homogenates (∿2 for Santa Barbara mussels and ∿40 for Alaskan mussels) are consistent with the suspected source of the hydrocarbons in each sample, i.e., biogenic for the Alaskan sample and petroleum for the Santa Barbara sample (Farrington and Meyer, 1976).

Table 2 Homogeneity Studies on Intercalibration Materials

	Alaskan Mussels		Santa Barbara Mussels	
Date of Analysis	Bottle	Hydrocarbons in GC range (μg/g)	Bottle	Hydrocarbons in GC range (μg/g)
10/77	A-6	42.1 7.8 19.0 23 ± 17.5 (76%)	S-3	38.3 12.3 25.3 ± 18.4 (73%)
11/77	A-21	27.7 28.9 31.5 29.4 ± 1.9 (6%)	S-17	10.8 31.5 30.1 28.3 25.2 ± 9.7 (38%)
12/77	A-28	59.9 57.9 61.9 50.2 57.5 ± 5.1 (9%)	S-11	58.9 44.6 41.2 48.2 ± 9.4 (20%)
1/78	A-3	69.9 59.4 45.3 58.2 ± 12.3 (21%)	S-23	47.1 59.1 54.3 53.5 ± 6.0 (11%)
5/78			S-42	29.6 44.0 37.9 41.8 38.3 ± 6.3 (16%)
5/78			S-49	49.4 52.1 56.5 42.3 50.1 ± 6 (12%)
Overall Average :		43.2 ± 18.9 (44%)		40.5 ± 13.7 (34%)
12/77-5/78 Average:		57.8 ± 8.0 (14%)		47.1 ± 8.5 (18%)

Table 3 Alaskan Mussels

	Total Hydrocarbons in GC Elution Range (µg/g)							Total Extractable Hydrocarbons (µg/g)	Pristane/ phytane
	Aliphatic			Unsat./Aromatic			Total		
Laboratory	Resolved	UCM*	Total	Resolved	UCM	Total			
1	4.4	14.8	19.2	8.8	29.9	38.7	57.8	139.1	58
2			--			--	--	--	40
3			--			--	41.1	73.3	40.5
			--			--	32.2	38.4	38.4
4	--	--	12.0	--	--	89.4	101.4	--	70.8
5	4.9	--	--			--	--	--	40.1
	5.2	--	--			--	--	--	39.7
6	1.5	--	--	2.1	--	--	--	--	--
	2.6	--	--	2.3	--	--	--	--	--
7	3.4	16.0	19.4			--	--	--	45
	1.5	22.0	23.5			--	--	--	--
8							43.2 ± 18.9		
Range	1.5-4.9	14.8-22.0	12.0-23.5	2.1-8.8		38.7-89.4	32.2 - 101.4	38.4 - 139.1	38.4-70.8

-- Data not reported.

*UCM = unresolved complex mixture in gas chromatogram.

Table 4 Santa Barbara Mussels

	Total Hydrocarbons in GC Elution Range (µg/g)							Total Extractable Hydrocarbons (µg/g)	Pristane/ phytane
	Aliphatic			Unsat./Aromatic			Total		
Laboratory	Resolved	UCM*	Total	Resolved	UCM	Total			
1	6.5	99	105.5	6.8	144	150.8	256.3	300.9	1.3
2	--	--	--	--	--	--	--	--	N.D
3			--			--	130.2	177.0	2.3
			--			--	148.4	162.9	1.8
4	--	--	42.7	--	--	15.4	58.1	--	11.1
5	2.88	--	--	--	--	--	--	--	1.96
6	1.8	--	--	<0.01	--	--	--	--	--
	1.52	--	--	0.76	--	--	--		
7	3.0	99	102	0.17	--	--	--	--	2.0
	1.9 ± 0.4	66 ± 5	67.9	0.08	--	--	--	--	2.1
8							40.5 ± 13.7		
Range	1.8 - 6.5	66-99	42.7-105.5	0.01-6.8	15.4-150.8		40.5 - 256.3	162.9 - 300	1.3 - 2.3

N.D. - not detected

-- Data not reported

*UCM = unresolved complex mixture in gas chromatogram.

The most abundant aliphatic hydrocarbons in the Alaskan mussels were pristane (2.7 ± 0.6 μg/g), n-pentadecane (0.27 ± 0.1 μg/g), and n-hexadecane (0.29 ± 0.1 μg/g). In the Santa Barbara homogenate pristane was also the predominant hydrocarbon (0.45 ± 0.06 μg/g), but it was not as abundant as in the Alaskan sample. The levels of the other hydrocarbons in the Santa Barbara mussels were similar to those in the Alaskan sample (nC_{15}: 0.25 ± 0.6 μg/g and nC_{16}: 0.26 ± .17 μg/g). The majority of the laboratories were in good agreement for individual compound quantitation, but, in both samples the ranges for some individual n-alkanes differed by as much as a factor of 10. The most abundant aromatic hydrocarbons were reported to be C_1-, C_2-, and C_3-substituted naphthalenes and C_1- and C_2-substituted phenanthrenes. Two laboratories reported trace amounts of pyrene and fluoranthene as the only PAHs larger than four rings (Santa Barbara mussels were ten times greater than the Alaskan mussels).

The results of this interlaboratory comparison indicate the variability of state-of-the-art hydrocarbon determinations in a tissue matrix. These results are encouraging when compared with the results of an earlier intercomparison on sediment analyses (Hilpert and coworkers, 1978) in which values often differed by more than an order of magnitude. A more detailed report of this tissue intercalibration study will be published elsewhere (Hertz and coworkers, 1979).

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Shipboard Intercalibration of Filters used in the Measurement of Particulate Organic Carbon

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ABSTRACT

Analysis of the results of 72 sets of intercomparisons between filter types shows that there is a statistically significant difference between the particulate organic carbon (POC) results taken with glass fibre filters and those taken with silver filters, and between the 0.45 μm and the 0.80 μm silver filters. Within a single water mass, rough conversions can be made between the amounts collected on the various kinds of filters. When several water masses are to be compared, a single filter type must be used throughout; it is at least possible that the relationship between filter types is a function of the particle size distribution in a given water mass, and is specific to that water mass.

Keywords - intercalibration, filtration, particulate organic carbon

INTRODUCTION

Various groups of investigators determining particulate organic carbon (POC) concentrations in the oceans have adopted techniques of sampling and analysis which are similar, but which nevertheless seem to lead to different results and conclusions. The possibility has existed that the differences in results were due to large real differences in particle size distributions in the regions sampled; however, when a consistent scheme of sampling and analysis was applied to the South Atlantic and to the North and South Pacific Oceans, the POC results were closely comparable (Wangersky, 1976). The large differences found in the literature which cannot be attributed to seasonal or strictly local influences must result from points of difference in the techniques of sampling or analysis.

Among the differences in techniques is the choice of filter; the early work (Riley, Wangersky, and Van Hemert, 1964) was done with the Gelman E filter, at that time the only glass fibre filter available, while the more recent work has employed either the Whatman GF/C glass fibre filter or the Selas Flotronic 0.8 μm silver filter. It has been tacitly assumed that these filters each remove about the same fraction of the particulate matter from seawater. The only comparative study of the efficiency of filtration of these materials (Sheldon, 1972) estimated the amounts and the size distributions of the particles removed by measuring particle size distributions before and after filtration. The amount actually removed was not measured, nor was enough material run through the filters to estimate the effect of clogging of the filter pores.

This study was designed to discover whether there are consistent differences in retention between the most commonly used filter types under normal conditions of use in the field. Accordingly, our interest lay in comparing the amounts retained from 5 l samples of seawater, gravity filtered, with the usual but no unusual precautions taken in the sampling procedure. A major difficulty in this line of research is the large amount of sampling time needed to establish the statistical validity of differences between filter types. Ideally, we would wish to compare filters over a wide range of POC concentrations, or, in this case, from waters of widely differing particulate concentration. This is particularly important when we are comparing the extent to which the various filter types will clog in regions of high particulate concentration. However, we were limited both in the area of the ocean and in the time of year in which we could obtain the necessary sampling time; in the northern North Atlantic, POC values are likely to be quite low at all depths during the winter months. We are therefore including in the results some comparisons between two of the filter types from an earlier cruise, covering a somewhat greater range of POC concentrations.

It was not possible, within the limited sampling time available, to compare every type of filter which has ever been used for the measurement of POC. We chose to compare the filter types most commonly used in these measurements, the Gelman E, the Whatman GF/C, and the Selas Flotronic 0.45 and 0.8 μm pore sizes. The Gelman and Whatman glass fibre filters offer only a nominal pore size, the quoted apparent pore size (0.3 and 1.3 μm respectively) referring to the size above which all particles are retained. As Sheldon (1972) has shown, these filters retain a considerable proportion of the smaller particles as well. The Flotronic silver filters, on the other hand, offer clearly defined pore sizes, as well as considerably greater ease of handling. However, they are expensive, and their effective pore sizes are not as clearly defined as the company literature would have us believe. As delivered from the manufacturer, the filters contain amounts of organic carbon which are large and variable in comparison to the POC content of oceanic waters. If the filters are heated in air or oxygen to remove this carbon, the pore size increases somewhat, according to the manufacturers. The 0.45 μm pore size is supposedly more affected by the heating process than are the 0.8 or 1.2 μm sizes. No real test of these suppositions has appeared in the literature; we have therefore decided to compare the retention of the treated 0.45 μm with that of the treated 0.8 μm, since these are the two most commonly used sizes.

When the component being measured is so close in size to the method blank, the manner in which the blank is calculated can become of major importance in the determination. In the measurement of POC, it has been demonstrated (Wangersky, 1974) that the determination of the blank is the largest source of variability in the procedure. There has also been no agreement on the proper method for the calculation of a POC filter blank (Menzel, 1966; Kinney, Loder, and Groves, 1971; Banoub and Williams, 1972; Loder and Hood, 1973; Gordon and Sutcliffe, 1974). The point in question has been the possible adsorption of dissolved organic matter by the filters; while this does not seem to be a problem with the silver filters, the two types of glass fibre filter tested have much more surface area. The amount of dissolved organic carbon (DOC) adsorbed could be great enough to affect the POC values reported. The most common method of calculating the amount of DOC adsorbed has been to pass the seawater sample through a stack composed of two filters, and to assume that all of the particulate matter was retained by the top filter. The increase above the blank value of organic carbon on the bottom filter was considered to be due entirely to adsorption of DOC, and the blank for the filtration process was taken as the amount of carbon found on this bottom filter. In some cases, a stack of three filters was used, the bottom filter was discarded as possibly contaminated, and the middle filter was taken as the blank for the process (Banoub and Williams, 1972). An attempt was made to investigate the size of the correction for adsorption to be expected in water masses of varying organic carbon content.

MATERIALS AND METHODS

The heterogeneity of the universe being sampled has made comparison of sampling efficiencies of various filters difficult. Sample bottles even 5 m apart may be sampling different universes, and a string of five sampling bottles, spaced as closely as is consistent with proper operation, may be spread over some 25 m. However, a Niskin rosette sampler can be rigged to release all of its samplers at one command from the surface, thus taking true replicate samples at a given depth (Wangersky, 1978). With such an apparatus, the variability in POC to be expected at a single point in the ocean at a single time has been determined (Wangersky, 1974). With silver filters of 0.8 μm pore size, the expected standard deviation in a cast of five replicates is 1.3 μg C/l.

The same apparatus can be used to study the differences between filter types. In comparative studies, the rosette is loaded with six or twelve 5 l Niskin bottles, and a group of the bottles are closed simultaneously. In the case of the older rosette, with which the work prior to 1978 was done, all six of the bottles on the rosette were tripped at a single depth. With the newer rosette, carrying twelve bottles, samples were taken in groups of four at each of three depths. Half of the samples from a single depth were then filtered through one type of filter, and half through the other type; the mean POC values from these two sets of filters were then compared. When enough casts had been made to ensure reasonable statistical reliability, a *t* test on paired samples was performed to determine whether the differences between the means of the paired sample sets were significant.

The older set of samples, comparing the GF/C filters with the Flotronic 0.8 μm silver filters, was tken on Dawson cruise 73-030, on the Halifax-Bermuda section, October, 1973. Station locations are given in Wangersky and Hincks (1978). Six 5 l samples were taken at each of two depths, 250 m and 500 m.

The more recent set of samples was collected on Hudson cruise 78-002, to the Labrador Sea. For each of the three pairs of filters, a minimum of 21 depths was sampled. The sampling was concentrated around 20 m, 200 m, and deeper than 500 m. Station locations for this cruise are also given in Wangersky and Hincks (1978).

Samples for the examination of the blank calculation were taken on a short cruise of the CNAV Bluethroat in September, 1972 and on the Halifax-Bermuda section on Dawson cruise 72-034 in October and November, 1972. Each sample was filtered through a filter pack consisting of a 47 mm diameter 0.8 μm pore size silver filter on top and a Whatman GF/C filter below, held in a 250 ml polycarbonate filter holder. The station locations and the results of the analyses of the silver filters have already been reported in Wangersky (1974). The glass fibre filters for the Bluethroat cruise were taken from one lot, and those for the Dawson cruise from another. The two lots of filters displayed significantly different carbon blanks, 25.9 μg C, SD = 2.6 for the Bluethroat filters, and 12.1 μg C, SD = 2.2 for the Dawson filters. The two lots were treated identically, being baked for 2 hrs at 450°C; we cannot explain the difference in blank values. The blank values were determined on filters which had been treated exactly like sample filters, even to being taken out to sea and inserted into filter holders.

The sampling and analytical methods used in the investigation of filter blanks has already been described (Wangersky, 1974). The same methods were used in the filter comparisons, except that an induction furnace was used for the combustion.

RESULTS

Filter Blanks

The data from the determination of organic carbon in the bottom filters are presented in Wangersky and Hincks (1978). In the main, our findings using glass fibre filters parallel those of Gordon and Sutcliffe (1974), who used silver filters throughout. At all stations, the 25 m samples displayed appreciable amounts of organic carbon on the bottom filters. Organic carbon values significantly greater than the filter blanks could be found down to 250 m at the Gulf Stream station. At all other stations, the carbon values for the bottom filters at depths greater than 25 m were for the most part indistinguishable from the filter blanks (average = 3.9 µg C/l, SD = 3.8). The glass fibre filters would thus appear to behave just like the silver filters.

If all of the data are combined, a correlation between carbon values on the top and bottom filters can be found, as was also observed by Gordon and Sutcliffe (1974). The correlation is highly significant ($r = +0.60$, $n = 211$), but highly misleading. An examination of the distribution of values for the top and bottom filters shows the data from the top filters to be bimodal (Figs. 1 and 2), and indicates that the data should be divided into two universes on the basis of depth. Samples taken at 25 m display a correlation between top and bottom filter values ($r = +0.42$, $n = 30$) significant only at the 5% level. The deeper samples show a correlation ($r = +0.10$, $n = 181$) which is clearly not different from random. Thus even in the surface samples the variability in the top and bottom POC values is only weakly related, and in the deeper samples the two filters vary independently.

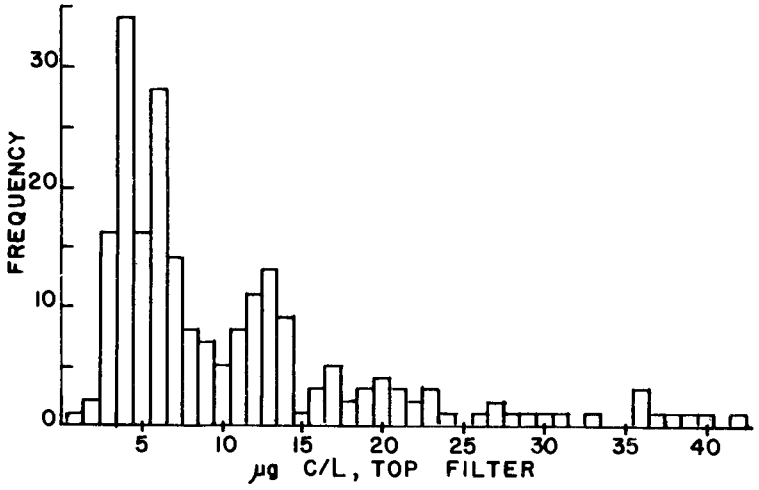

Fig. 1. Frequency distribution, POC, top filter, silver, 0.8 µm

If the regression between top and bottom filters from surface samples is extrapolated back to the blank value for the bottom filters, the regression line intersects the axis at a value of 30 µg C, essentially the same value found by Gordon and Sutcliffe (1974). It would thus appear that the processes leading to the accumulation of organic carbon by the bottom filters are much alike for glass fibre and silver filters.

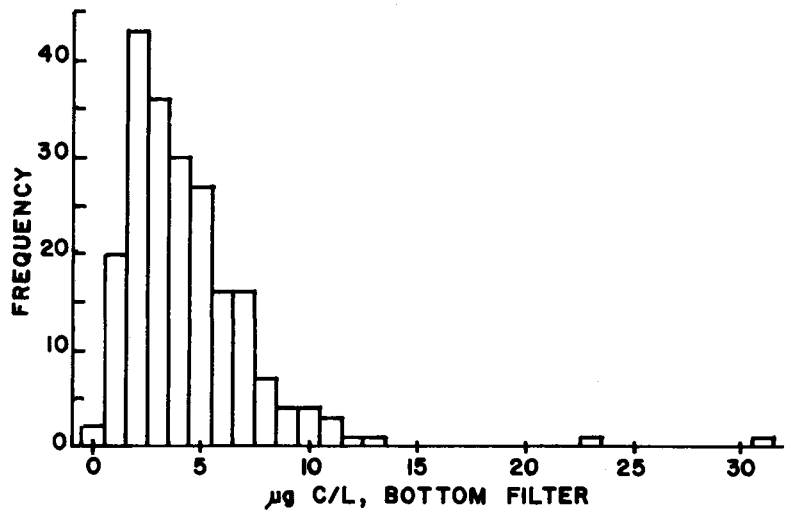

Fig. 2. Frequency distribution, POC, bottom filters, glass, GF/C

The mechanism of organic accumulation on the bottom filter is obscure. Since filter packs of three or more silver filters invariably showed no organic carbon in the intermediate filters (Gordon and Sutcliffe, 1974), it seems unlikely that the bottom filters in their experiments were simply catching smaller particles escaping the top filters. Simple adsorption of dissolved material could also be ruled out. In our experiments, the occasional accidental introduction of a second glass fibre filter into a stack similarly gave evidence of no adsorption of dissolved organic matter. While the accumulation on bottom filters must be related to the composition of the organic carbon in surface waters, the nature of this relationship requires further study.

The interpretation of the carbon content of the bottom filter is therefore open to considerable question, for both the silver and the glass fibre filters. Since the POC is defined operationally as the material caught by a filter of a certain pore size, rather than by any natural discontinuity in the distribution of particle sizes in the ocean, the "correction" for adsorbed organic carbon would be questionable even if it were understood. Therefore, we determined our filter blanks as the amount of organic carbon measured on filters taken through the whole process of filter handling, but with no seawater passed through them.

Comparison of Filter Types

The POC data used in these comparisons of filter types can be found in Wangersky and Hincks (1978). The statistical comparisons are presented in Tables 1 and 2. It can clearly be seen that the glass fibre filters, in spite of their nominally larger pore size, collected more POC than did the 0.8 µm silver filters. The 0.45 µm silver filters also collected more POC than did the 0.8 µm filters.

Each data set was subjected to a t test for paired data (Table 1). The difference between the Gelman and the Whatman filters was not significant, but the differences between the Whatman GF/C and the 0.8 µm silver filters, and between the 0.8 and 0.45 µm silver filters, is clearly significant. The different filter types

and sizes select slightly different portions of the environment.

Table 1 Comparison of Filter Sets

Filter	D.F.	t (paired)	Significance level
GF/C vs. Gelman	26	0.88	0.20
GF/C vs. 0.8 μm silver (first set)	13	11.17	< 0.001
(second set)	20	3.50	0.005
0.8 μm silver vs. 0.45 μm silver	20	3.45	0.005

DISCUSSION

The Calculation of Blanks

The use of the bottom filter as a blank for the top filter, in the usual method of non-replicated sampling, would result in subtle differences in the values reported for POC and in their interpretation. In the surface waters, the POC values would be diminished, generally by 10-15%. Given the normal distribution of surface values, ranging from 25-100 μg C/l, depending upon the region and time of year, this difference would not be apparent; in most cases, it would be lost in the local variability. In the deeper water, there would occasionally be samples whose blank value would exceed the top filter value; these samples would probably be discarded as contaminated.

The major effect of this method of calculation for filter blanks is an increase in the apparent variability of the deep values due to the greater variability of the blanks. The assignment of this increased variability influences our concept of the distribution of POC in the deep ocean. If it is considered that this variability is an attribute of the sampling and analysis, the variations in POC content to be found in the deep ocean all lie within the analytical error of the method, and the deep ocean appears to be a basically homogeneous universe. This view of the nature of the deep ocean is reinforced by the practice of reporting POC values as averages of all samples taken at a given depth from a single area. If, on the other hand, the blank value is calculated from filters which have not had seawater passed through them, a large part of the variability in POC will be assigned to the environment. The consequences of this assignment of variability have been examined in earlier papers (Wangersky, 1974, 1976, 1977, 1978).

This work has led us to believe that the variability is properly assigned to the environment; simple adsorption of dissolved organic matter does not seem to be a sufficient explanation for the extra organic matter on the bottom filter of samples from surface waters. Banoub and Williams (1972) have suggested that the bottom filter be used as a guard against contamination arising from the filter holder. They discard the bottom filter before analysis. This technique results in a considerable increase in filtration time when gravity filtration is employed. We have preferred to use gravity filtration in order to minimize damage to organisms during processing; therefore we have continued to use the single-filter technique.

Comparison of Filter Types

While the first set of comparisons of the GF/C and the 0.8 μm silver filters, involving samples from three quite different water bodies, the slope water, the Gulf Stream, and the Sargasso Sea, displayed no simple relationship between the sampling efficiencies of the two types of filters (Fig. 3), the later work, drawn from a single region of the ocean, showed relatively strong linear relationships between each of the pairs of filters tested.

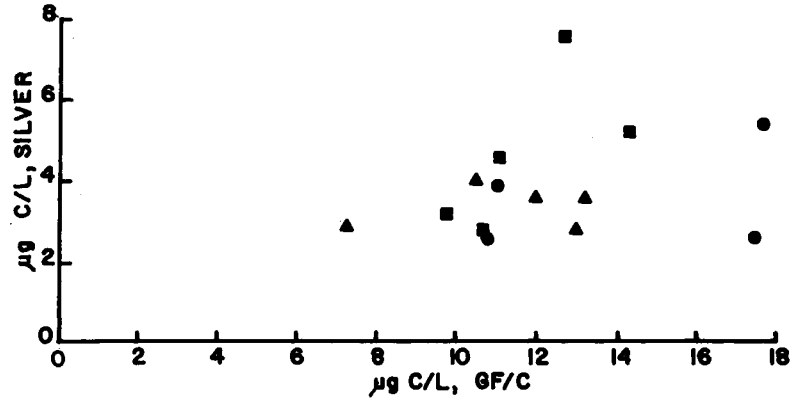

Fig. 3. POC, silver 0.8 μm filter vs. GF/C, Dawson cruise: OOO, slope water; ΔΔΔ, Gulf Stream; □□□, Sargasso Sea

Since the filters in any single comparison must both be considered to be independent variables, with the degree of variability to be expected from environmental samples, there is not just one simple relationship between any two filter types. Rather, the slope and intercept of the regression line depend upon which variable is taken as the independent variable (Figs. 4, 5, 6). The theoretical basis for correlations between two independent variables is still rather tentative, and the assignment of a correlation coefficient based on a least squares regression is only approximately correct, at best. However, the coefficients are sufficiently high that there is little doubt that the relationships are real (Table 2).

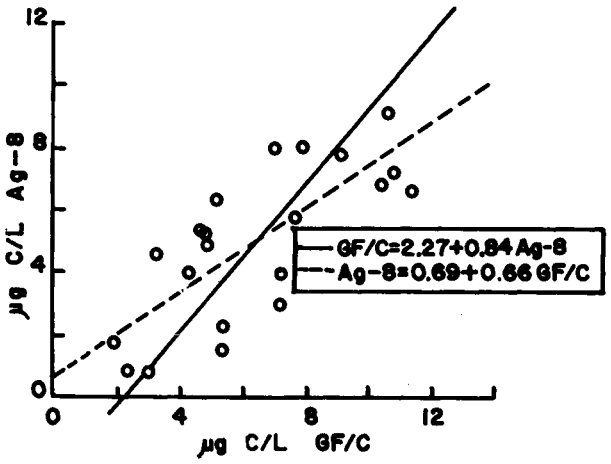

Fig. 4. POC comparison, GF/C vs. silver 0.8 μm

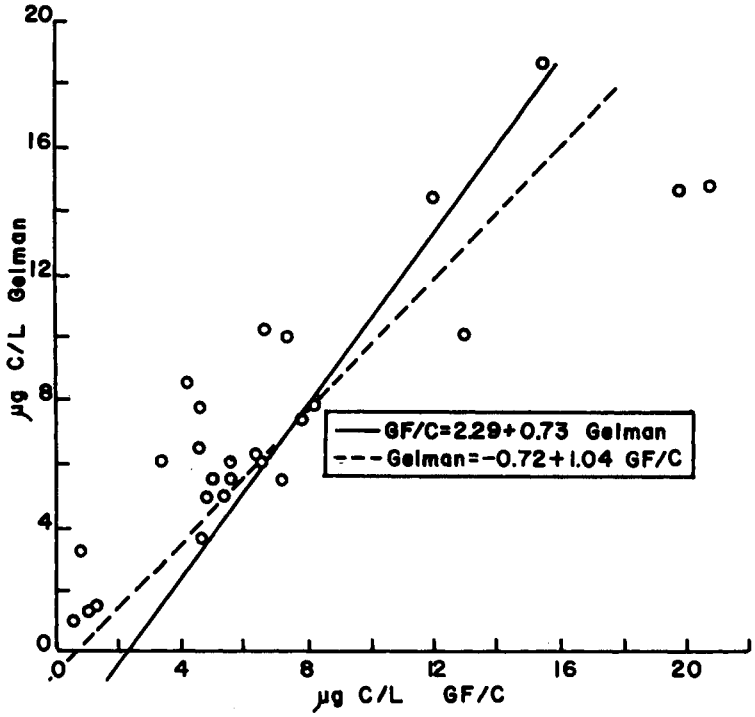

Fig. 5. POC comparison, GF/C vs. Gelman

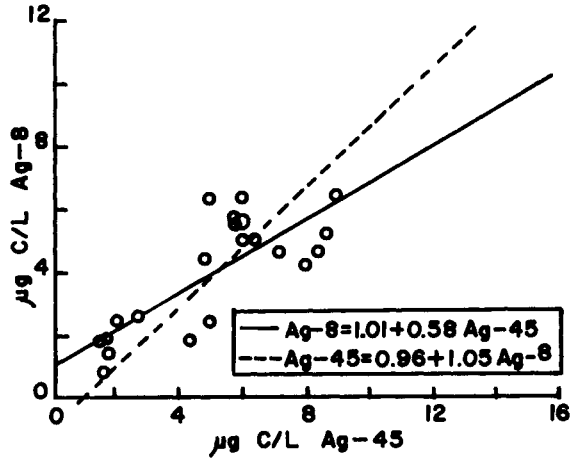

Fig. 6. POC comparison, silver 0.8 µm vs. silver 0.45 µm

The comparisons between filter types which have been made in this investigation suggest that within a single region of the ocean it is possible, within limits, to convert the data taken with one type of filter to a form which can be compared with that taken by another type. Unfortunately, in this series of samples we did not find regions very high in POC; therefore, the effects of heavy particle loading could not be investigated. At the low POC values to be found in the North

Atlantic in winter and in the deep ocean everywhere, the two silver filters are clearly different from each other and from the glass fibre filters in filtering efficiency. The two glass fibre filters tested are only marginally different.

Table 2 Correlations between Filter Types

Filters compared	r	n	Significance level	Average ratio A:B
Gelman:GF/C	0.87	27	<0.001	1.15
GF/C:0.8 μm,				
first set	0.27	14	n.s.	3.37
second set	0.74	21	<0.001	1.61
0.8 μm:0.45 μm	0.78	21	<0.001	0.76

These comparisons between filter types should be continued into regions of higher POC content. We would predict that at higher particle loadings the filters will give substantially the same values; the relationships between filter types would not remain linear. In the regions of high POC content, comparisons should also be made at several filtration volumes, to establish rates of pore blocking for each type of filter.

The problem of possible differences in filtration efficiencies in different water masses is not as simply resolved. In order to establish statistically valid differences between water masses, enough comparisons must be made in each water mass to establish regression lines for each pair of filter types. The number of samples and the wire time required make this a project to be pursued on cruises of opportunity over many years. Alternatively, a particle counter extending to the 0.2 μm range might be used, although there is no assurance that particle concentration and POC are necessarily closely correlated in the same fashion everywhere in the oceans. What started out to be a relatively simple problem in sampling technique has thus turned into an inquiry on the nature of particle size distributions in different water masses, and the manner in which these distributions affect the apparent POC values taken from these water masses on filters with different filtration characteristics.

We would emphasize the "apparent" nature of the POC so determined because we are actually sampling the particle size distribution after some of the heavier particles have settled to the bottom of the sampling bottle, and after the original particles have been run through a sampling bottle valve. The size of the aperture in the valve should set the upper limit for the size of any single particle, while the turbulence associated with the draining of the bottle should break up any fragile particulate associations. What we are really measuring in our POC determinations is a slice of the particulate distribution whose upper limit is set by the geometry and physics of the sampling system, and whose lower limit is set by the choice of filter; "apparent" would seem to be the proper term for the resulting numbers. There is no *a priori* reason to expect that any quantity so determined should correspond to any property of the water masses which is of importance to the organisms living there.

CONCLUSIONS

1. The single-filter technique of sampling, with gravity filtration, should be adopted as standard.

2. One single type of filter, and one porosity, should be used for all sampling.

This is especially important in any study involving several water types.

3. The silver filters are to be preferred over the glass fibre filters because of their greater uniformity in pore size. The 0.8 µm pore size is better than the 0.45 µm size, because of the better replication (for the 0.45 µm filters, the difference between replicates averaged 1.45 µg C/l, SD = 1.76; for the 0.8 µm size, 0.96 µg C/l, SD = 0.63).

4. If glass fibre filters must be used, the Whatman GF/C filters (average difference between replicates is 2.00 µg C/l, SD = 1.88) are slightly more uniform than the Gelman filters (average difference is 2.34 µg C/l, SD = 2.50).

5. The possibility that differences between water masses in POC size distributions are great enough to affect the relationships between POC values determined using different filter types should be investigated.

ACKNOWLEDGEMENTS

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Determination and Identification of Hydrocarbon Pollutants by Thin-layer Chromatography

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ABSTRACT

Non-volatile hydrocarbons in a marine sediment extract have been determined by thin-layer chromatography (TLC) with precision approaching that obtainable by gas chromatography. The method involves chromatography of chloroform extracts on silica gel plates using hexane as a solvent. The hydrocarbons are charred and quantitated photodensitometrically. By use of a mixed alkane standard and a mixed aromatic standard for calibration, the saturated hydrocarbons, unsaturated hydrocarbons, and total nonvolatile hydrocarbons were determined with precisions of 8-12%, relative standard deviation. Elemental sulfur, traces of chlorinated hydrocarbons, and phthalate esters do not interfere and need not be removed. This thin-layer chromatographic method is also useful for oil spill source identification. A South Louisiana crude, a Kuwait crude, a Venezuelan bunker fuel, and a No. 2 fuel oil were easily distinguished by their thin-layer chromatograms. The chromatograms were only minimally affected by weathering of the oils. The TLC "fingerprinting" of waterborne oils was proven in a blind test with 4 pairs of oils.

Key words: Thin-layer chromatography, hydrocarbons, alkanes, arenes, sediments, oil fingerprinting.

INTRODUCTION

Quantitative analysis of hydrocarbon pollutants is important for the assessment of the fate and effects of petroleum in the marine environment, and qualitative analysis of petroleum spills is required for source identification. High-resolution techniques including capillary gas chromatography (May and co-workers, 1975) and gas chromatography/mass spectrometry (Eglinton, Simoneit and Zoro, 1975) are useful for analysis of environmental hydrocarbons. The equipment required for these analyses is expensive and preseparation of hydrocarbons into classes is necessary. Rather than attempting such detailed identification of individual hydrocarbons, our studies have focused on measurement of total saturated hydrocarbons (alkanes) and total unsaturated hydrocarbons (predominately arenes). Elevation of hydrocarbon levels is taken as a criterion of appreciable pollution. A method for determination of total nonvolatile alkanes and arenes

by thin-layer chromatography (TLC) has been developed in our laboratory (Hunter, Guard and DiSalvo, 1974; Hunter, 1975). This method is simple, inexpensive and requires no preseparation for analysis of sediment and water extracts. Recent improvements in this method and its application to oil source identification will be discussed.

EXPERIMENTAL

Materials

"Distilled in glass" chloroform and hexane (u.v. grade) were obtained from Burdick and Jackson Labs, Muskegon, MI. Other solvents were reagent grade. Silica Gel G plates (Analtech Uniplates) with a 250 μ layer of silica gel without organic binder were used after precleaning with acetone or ethanol and air drying. South Louisiana crude oil, Venezuelan bunker fuel, Kuwait crude oil and a No. 2 fuel oil were supplied from American Petroleum Institute Standards by Jack Anderson, Texas A&M University. Weathered samples were obtained by heating the oil under nitrogen until the residue reached a temperature of 288° C. For the blind matching test, 8 paired waterborne oil samples randomly labeled 1-8 were obtained from the United States Coast Guard. Subsequent to analysis, the samples were identified as follows: 1. and 4., crude oil from Santa Barbara seep; 2. and 8., waste gasoline and lube oil; 3. and 6., storm drain effluent-lube oil and vegetable oil; 5. and 7., coconut oil.

Quantitative Determination of Hydrocarbons

Sediments were collected by U.S. Army Corps of Engineers personnel, transferred to chloroform washed metal containers, and stored at 4° C until analysis. Sediment samples were lyophilized until dry ca. 24 hr and were extracted with 150 ml chloroform by grinding with a mechanical high-frequency homogenizer (Tekmar Tissuemizer) for 1-2 min. The solvent was evaporated on a rotary evaporator. The residue was dissolved in chloroform, and the volume was adjusted to 1.00 ml. Small amounts, 2μl, were spotted on Silica Gel G plates. At least 3 amounts of the alkane standard and the aromatic standard were spotted. The plates were developed with hexane. Visualization was accomplished by charring. The plates were sprayed with 0.25% (w/v) $K_2Cr_2O_7$ in conc. H_2SO_4 and heated to 250° C for 2 hr. The plates were scanned with a photodensitometer and connected to a log recorder. The areas of the alkane peaks and under the aromatic envelope were measured by weighing xeroxed copies. The amounts of alkanes and aromatic hydrocarbons in each sample were calculated from standard curves determined on the same plate. The alkane and arene standards were prepared by liquid chromatography of a mixture of 0.1 ml South Louisiana crude oil, 0.1 ml Kuwait crude oil, and 0.14 ml Venezuelan bunker fuel on silica gel.

Oil Identification

Oil samples were collected by U.S. Coast Guard personnel in glass jars fitted with Teflon-lined lids or using fiberglass mats. Samples were refrigerated upon arrival at the laboratory until analysis. Oil was recovered by extraction with chloroform, or by repeated dipping of a glass pipet through the oil-water interface. Solutions containing 50 mg/ml of oil in chloroform were prepared. A small amount (1.0 μl) of chloroform solution was spotted on a Silica Gel G thin-layer plate. Thin-layer chromatography, visualization and photodensitometry were carried out as described above.

Peak heights were calculated relative to the tallest peak, whose height was defined equal to 100. The R_{st} values were calculated relative to the alkane peak, whose R_{st} value was defined as 100. Standardized "fingerprints" were obtained as bar graphs of relative peak height vs R_{st} value. The bars were connected to aid visual pattern recognition.

RESULTS AND DISCUSSION

Thin-layer chromatography of hydrocarbons on silica gel is a low-resolution technique with the capability for separating saturated hydrocarbons, unsaturated hydrocarbons, and polar compounds including triglycerides. When using hexane as a solvent and visualizing by charring, the saturated hydrocarbons (alkanes) appear as a spot, with a typical R_f value of 0.78 (Fig. 1). The unsaturated hydrocarbons (primarily arenes in petroleum oils but including naturally-occurring alkenes) appear as a streak from the origin to the alkane spot; whereas, at the other extreme the linearly fused ring polynuclear aromatics appear near the origin.

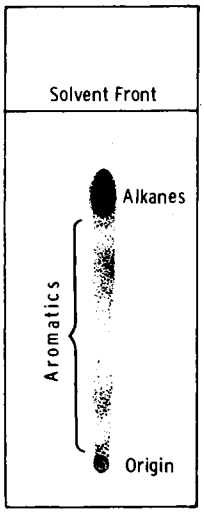

Fig. 1. Thin-layered chromatogram on silica gel. Solvent: hexane.

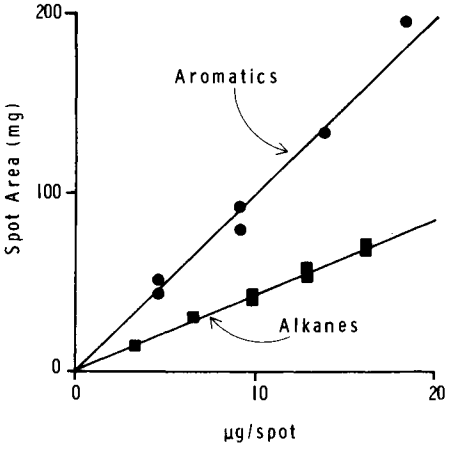

Fig. 2. Calibration curves for alkane and arene standards.

Microgram quantities of environmental hydrocarbons can be determined by thin-layer chromatography using direct photodensitometry of charred spots. Since various hydrocarbons respond differently to charring, it is important to select appropriate standards for quantitative comparison. Ideally the standard and the unknown should have the same composition but this is often impractical. Therefore, we have employed a mixed alkane standard and a mixed aromatic standard. The response curves for these standards are shown in Fig. 2. Charring is nearly twice as sensitive for aromatic hydrocarbons as for the alkane mixture.

The analytical precision of the determination of a sediment hydrocarbon mixture is shown in Table 1. Replicate single-plate determinations of alkanes, arenes, and total hydrocarbons were accomplished with relative standard deviations of 2.4%, 2.4%, 1.3%, respectively. Eight unknown samples plus the required 6 standards can be analyzed on a single 20x20 cm plate. Replicate plate determin-

ation of sediment hydrocarbons is less precise with relative standard deviations of 12%, 16% and 8% for alkanes, arenes and total hydrocarbons, respectively. These results indicate that with appropriate standards the TLC method offers a precision comparable to gas chromatographic methods. For comparison, an inter-laboratory relative standard deviation of 25% has been reported for determination of environmental hydrocarbons by gas chromatography (Hilpert and co-workers, 1978).

The results of a comparison of TLC analyses and gravimetric analyses by the method of the Am. Public Health Assoc. (1975) are presented in Table 2. These sediment extracts contained appreciable amounts of sulfur, a portion of which was included in the standard method hydrocarbon result. Therefore, the extracts were desulfurized by the method of Blumer (1957). Comparing the TLC result to the gravimetric determination after sulfur removal gave relative errors ranging from 18% to 45% indicating that the choice of standards results in high values for total non-volatile sediment hydrocarbons.

TABLE 1 Analytical Precision of Sediment Hydrocarbon Determination

	Hydrocarbon Content µg/g dry wt., $\bar{X} \pm SD$		
	Alkanes	Arenes	Total
Single Plate	333±8	123±3	457±8
(relative SD)	(2.4%)	(2.4%)	(1.3%)
Replicate Plates	336±41	111±12	448±36
(relative SD)	(12%)	(11%)	(8%)

TABLE 2 Analytical Accuracy of Sediment Hydrocarbon Determination

	Total Hydrocarbon Content µg/g dry wt., (ppm)			
Sediment	TLC Method	Std. Method	Desulfurized	% Error
Duwamish River	486±17	854	413	18
Bay Ridge	6030±406	5930	4145	45
Perth Amboy	1995±235	2850	1580	26
			Average % Error =	30

A major advantage of this TLC method stems from lack of interferences. Sediment and water extracts may be analyzed directly. Sulfur in extracts of anoxic sediments does not char. Triglycerides remain at the origin and are not measured. Traces of chlorinated hydrocarbons such as DDT and lindane (hexachlorocyclohexane) do not char. Common laboratory contaminants such as dibutyl phthalate (R_f=0.01) also remain near the origin and may be neglected. Tissue extracts, however, contain such large amounts of triglycerides that saponification and preliminary clean-up are required to prevent overloading.

Thin-layer chromatography with visualization by sulfuric acid charring provides

a rapid, simple method of oil spill identification when a high-resolution technique is not required. Samples of 30-60 mg are optimal with at least 0.3-0.6 mg required. The samples may be wet and contain sediment. The effect of weathering on the "standardized fingerprints" of 4 oils is minimal (Fig. 3). Statistical comparison of the 8 samples using a χ^2 test indicates significant differences between oil types, but not between weathered and unweathered samples at α=0.5.

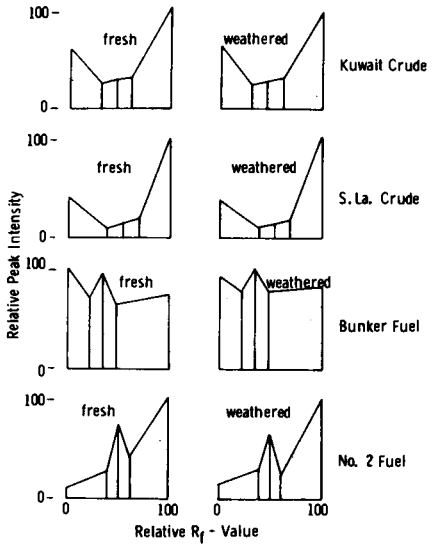

Fig. 3. Standardized "fingerprints" of fresh and weathered oils.

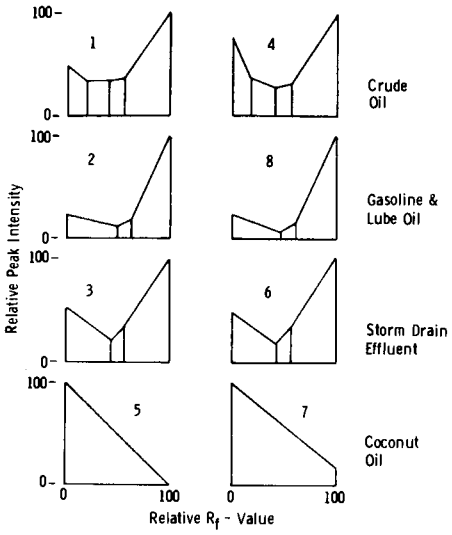

Fig. 4. Standardized "fingerprints" of oil samples in a blind test.

The results of a blind matching test are shown in Fig. 4. The pairs of oil samples were easily matched as: 1, 4; 2, 8; 3, 6; and 5, 7. These results were confirmed by the U.S. Coast Guard personnel who had prepared the samples.

In summary, oil "fingerprinting" by thin-layer chromatography with visualization by charring provides a method for oil spill identification that requires no pretreatment of samples, exhibits minimal differences as a result of the extent of sample weathering, and provides results amenable to mathematical ranking. The method has wide application in oil spill cases where the compositions of the oils differ by enough to allow the use of a low-resolution technique.

ACKNOWLEDGEMENT

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Fingerprinting Petroleum Pollutants in the Mediterranean Sea

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ABSTRACT

To confront the increasing and diversified petroleum pollution problems in the Mediterranean Sea there is a need to develop efficient analytical methods for the source identification of oil pollutants. The paper describes an analytical approach for fingerprinting crude oils from different geographical areas, which are of particular incidence in the Mediterranean, and gives special attention to the identification of chronic pollution samples from the open sea.

The approach involves, in a first step, the determination of the S, Ni and V contents of the samples as well as the phytane/pristane ratio from the HRGC profiles (FID). With this information a general assignment of the type of the pollutants and the area from where they come can be established. The precise identification of the samples source is attempted by a multi-fingerprinting procedure which is carried out by the use of selective detectors in GC. Four profiles are considered, corresponding to total and polyaromatic hydrocarbons (FID), sulphur (FPD) and nitrogen (NPD) compounds. Alternatively, the use of mass-fragmentography (GC-MS-COM) to obtain profiles for specific series of hydrocarbons of geochemical significance, such as C_{20}-C_{40} acyclic isoprenoids, C_{27}^{+} steranes and triterpanes is highly stressed.

KEY WORDS

Isoprenoids. Mass-fragmentography. Mediterranean Sea. Oil fingerprinting. Petroleum pollution. Selective GC deterctors. Steranes. Tar balls. Triterpanes.

INTRODUCTION

The Mediterranean Sea is among the first marine regions to show the symptoms of oil impact. In fact, the observed concentrations of petroleum tars on its surface are one order of magnitude higher

than those generally found in any other regional seas (Nat. Acad. of Sci., 1975). The factors that contribute to this situation are several. Thus, the particular hydrogeological conditions of the basin are such that oil entering or discharged there has little chance of leaving; it will stay and accumulate until it is degraded. Moreover, the cyclonic drift of the water circulation tends to deposit the oil on the shores or to accumulate it at certain exposed points. The oil handling activities brought about in the area, and specially the tanker traffic, are, in turn, quite important, as it is shown in Fig. 1. Finally, a lenient legislation has been unable to prevent intentional pollution.

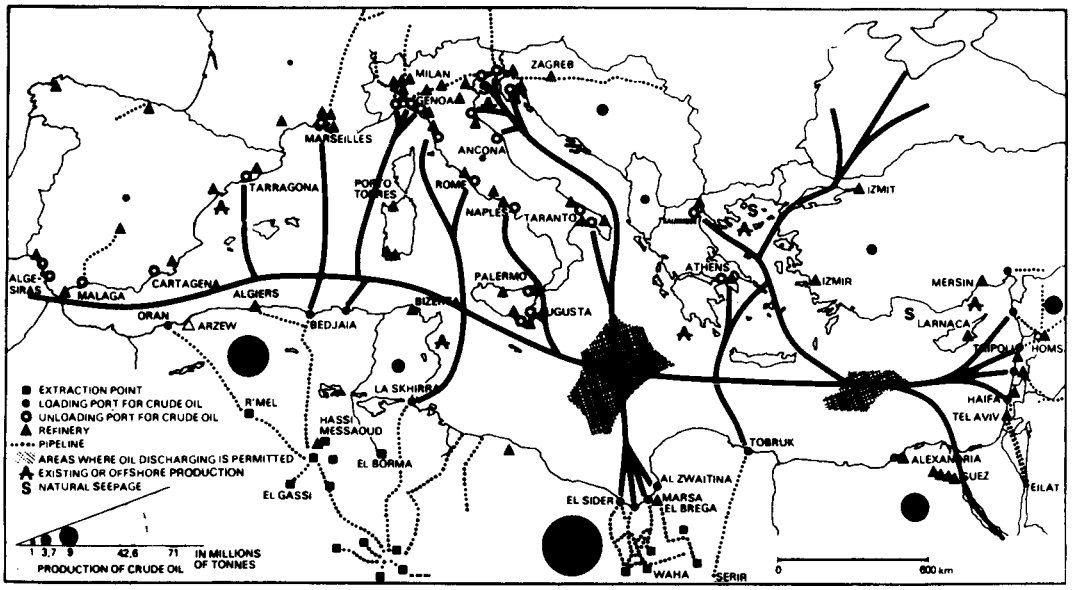

Fig. 1. Location of the different sources of petroleum pollution in the Mediterranean Sea. (courtesy of P. Le Lourd, 1977).

From all of the above, the oil pollution control in the Mediterranean has become a problem of mayor concern and consequently the development of analytical methods for the source identification of petroleum pollutants has received particular interest.

However, apart from the primary characterization of acute incidents, much attention has been recently devoted to the ultimate fate of spilled oils, specially for the assesment of their persistance into the sea and their contribution to the chronic pollution. Chronic pollution by oil is, in the Mediterranean Sea, far more important than accidental pollution and it is generally assumed that is equally due to marine operational losses and to land-based discharges, including petroleum, petrochemicals and fossil fuel combustion products. In these situations the chemical composition of the samples found in the sea can be considerably altered by environmental conditions. Therefore, the identi

fication methods of oil pollutants should be able to deal with an entire spectrum of products of different origins and ages.

The present paper gives an account of the analytical approach used for fingerprinting oil products of different sources carried throughout the Mediterranean, with special reference to the identification of highly weathered samples. This has been attempted by mass-fragmentographic fingerprinting of geochemically significant series of hydrocarbon components (biological markers).

EXPERIMENTAL

Reference samples of crude oils from Venezuela, Spain, Nigeria, Gabon, Libya, Algeria, Kuwait, Saudi Arabia, Iran, Irak, Oman and AbuDhabi were supplied by several refineries and production companies. Tar ball samples were collected during a survey cruise (july 1977) between Cartagena (Spain) and Civittavechia (Italy), with a neuston net. Samples were dissolved in toluene to eliminate extraneous materials and stored at -4ºC until analysis.

Total sulphur content was determined by combustion (ASTM D-129; Bomb Method) and Ni and V by flamless atomic absorption spectrometry (Perkin Elmer 4000, equipped with a graphite HG-74 furnace).

Dual FID/FPD and FID/NPD chromatograms were obtained by splitting the column effluent. The former profiles were run on the deasphaltened oil residues in n-pentane (40 volumes) and the latter on the "polar oil fraction", which was isolated by partition of the deasphalted fraction into a cyclohexane-nitromethane mixture (1:5). The gas-chromatograph (Perkin-Elmer 990) equipped with FI, FP and NP detectors was operated either with 9ft x 1/8" packed columns (1% Dexsil 300 or 3% OV-101 on Gas-Chrom Q 100-120) from 150 to 300ºC at 6ºC/min. or with 200ft x 0.02" capillary columns (OV-101) from 120 to 280ºC at 6ºC/min., using He as carrier gas.

Mass-fragmentography (MF) was performed on a LKB 9000 S/PDP 11 E 10 computerized GC-MS system. The injector and jet separator were maintained at 290ºC and spectra were recorded and disk stored at 4 sec. intervals. In this case, the "branched-cyclic fraction" was preferably used. This was isolated throuhgout the previous recovery of the saturated fraction by conventional silica-gel adsorption chromatography, with an absorbent-sample ratio of 20 (eluting solvent: n-penatne), and subsequent inclusion in 30 fold excess of 5Å molecular sieves (solvent: iso-octane).

RESULTS AND DISCUSSION

All identification methods of oil pollutants are essentially a matching of samples based on the geochemical principle that no two oils have identical compositions unless they have identical histories. Thus, in theory every oil product is unique; however, oil is a very complex mixture and anlysis is never complete, so

that very similar oils may appear to be identical. Furthermore, exact correspondence of compositions of samples exposed to different environmental histories cannot be expected.

As there is no single analytical technique that can fully characterize an oil product, identification must be established by a series of analyses involving gecohemically characteristic and weathering-resistant sample parameters. From the several techniques used for that purpose and according to our previous experience (Albaigés and others, 1976, 1979) we have selected the parameters indicated in Fig. 2 for building-up an inventory of possible source-oils in the Mediterranean, with which to compare the pollution samples. All of these parameters have been suggested as the most suitable for a quick screening procedure of the pollutants (Brunnock and others, 1968; Zafiriou and others, 1973; Shekel and Ravid, 1977). A major advantage is that the information required is already available for most of the crude oils or is easily obtainable by routine analytical methods, and although the values reported in the literature are referred to unexposed oils, their correlation with pollution samples can be attempted, because the effects of weathering can be predicted (Brunnock and other,1968; Blumer and other, 1973). The HRGC pattern (FID) from which the phytane/pristane ratios are obtained permit, in addition, to assess the type of pollutant, namely, oil sludge, crude oil,technical fractions, etc.

As it can be seen in Fig. 2, this characterization procedure allows a primary classification of the samples into several geographical groups. In this manner, we have assigned to Middle East (M.E.) sources 86% of the pelagic tar ball samples collected during a survey cruise in the Western Mediterranean (Albaigés and others, 1979). However, this classification apparently leads to a certain overlaping and, in agreement with Jeffrey and others (1974), it is unlikely that with such parameters one could be able to ascertain the specific origin of the samples. Their variation between crudes of the same area and their resistance to the sea weathering processes are not enough, in many cases, for providing the unequivocal identification of the pollutant. Furthermore, this procedure cannot be applied to hydrocarbon samples found in highly dispersed forms, namely dissolved in water, adsorbed in sediments or biological samples, etc.... Thus, to provide furhter insights into these problems the composition of the samples should be better defined, and since the analysis of such chemically complicated samples is so extremely demanding, the great potential of GC can be particularly useful.

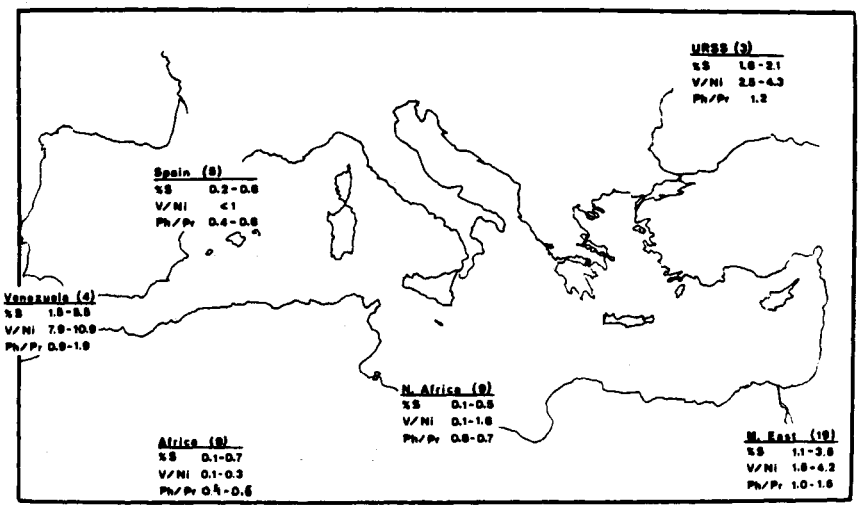

Fig. 2. Chemical charaterization parameters of crude oils handled in the Western Mediterranean. Numbers in brackets indicate the number of oils examined.

It is known that the GC traces of oil residues are characterized by a large unresolved envelope above the baseline, with small resolved peaks superposed. Incidentally, the occurrence of this unresolved "hump" in sediment or biota samples has been generally considered as an indication of petroleum contamination, being however its origin unknown. In order to get more information on the compounds included in this "hump" different types of detectors capable to enhance the response of specific series of compounds can be used. Fig. 3 shows a set of profiles actually measureble on petroleum residues. In addition to the already mentioned hydrocarbon (HC) fingerprint (FID), profiles of sulphur and nitrogen containing compounds have been obtained with the use of FP and NP detectors, respectively. In this manner, a multiprofile characterization procedure can be set up.

While the sulphur and HC profiles can be concurrently obtained straight from the samples by FPD and FID (Adlard and others, 1972), the smaller abundance of nitrogen compounds in unused oils requires their previous concentration. For this reason, since the applied concentration procedure consisted in a simple liquid-liquid extraction of the "polar oil fraction", polyaromatic hydrocarbons (PAH) were also extracted together with the N-containing compounds. This allowed the obtention of another representative FID profile (labeled FID(PAH) in Fig. 3) which will be useful in assigning the origin of the pollution in some chronic situations. If the source of HC is predominantly petroleum, the chromatogram will contain a large number of peaks arising from alkylated PAH, whereas if the source is pyrolytic (air pollution) or coal tar

or creasote in nature, a more simple profile will be observed, corresponding to a higher predominance of the unsubstituted species over their alkylated homologs (LaFlamme and Hites, 1978).

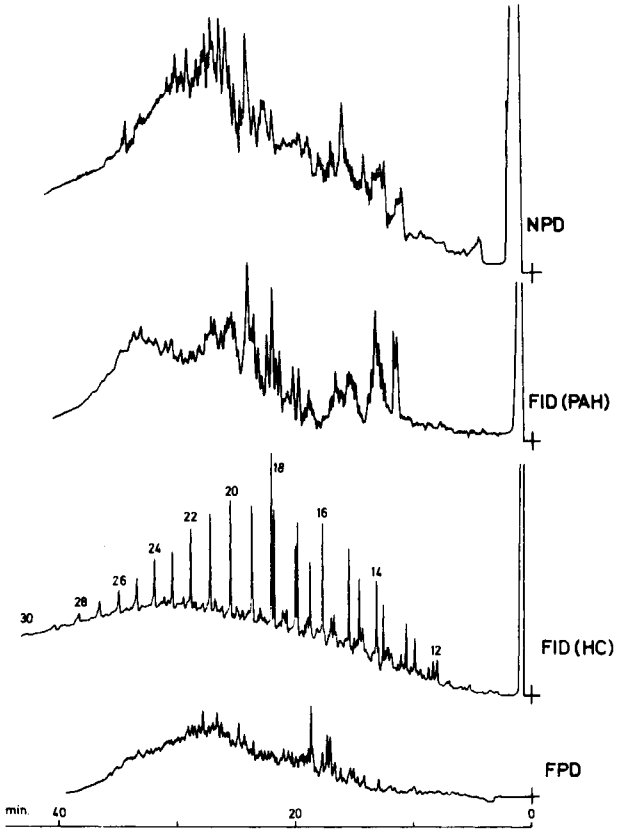

Fig. 3. GC profiles displayed by a Venezuelan crude oil residue (b.p. 200º C). Numbers over the peaks indicate the n-paraffin carbon atoms. FID (HC)/FPD profiles were obtained in a OV-101 capillary column, whilst the FID(PAH)/NPD in a 3% OV-101 packed column.

This fingerprinting procedure appeared quite succesful for the correlation of recent oil spillages with their suspected sources. Nevertheless, there were some question about the general specificity of these profiles and their stability throughout the environmental exposure of the product at the sea. Both aspects are of

interest in relation to pollution problems in the Mediterranean Sea, where, e.g., a particular incidence of very similar oil products from the M.E. area and a wide occurrence of weathered pelagic tars resulting from tanker washings are expected. Therefore a further evaluation of the method would seem in order.

The most significant chromatographic profiles are those displayed by the FID(HC) and FPD. Nevertheless Figs. 4 and 5 show the example of oils of similar geological origin, exhibiting sufficiently similar patterns to render difficult the precise identification of the pollutant source. The Aramco and Kuwait oils are apparently indistinguishable on the basis of the FID(HC) chromatogram and their FPD profiles exhibit only slight differences, being the latter very similar to other M.E. oils. The NPD and FID(PAH) patterns are of more limited value for oil identification because of the smaller variability among products, apart from the more time consuming procedure, as it has been recently noticed by Frame and others (1979).

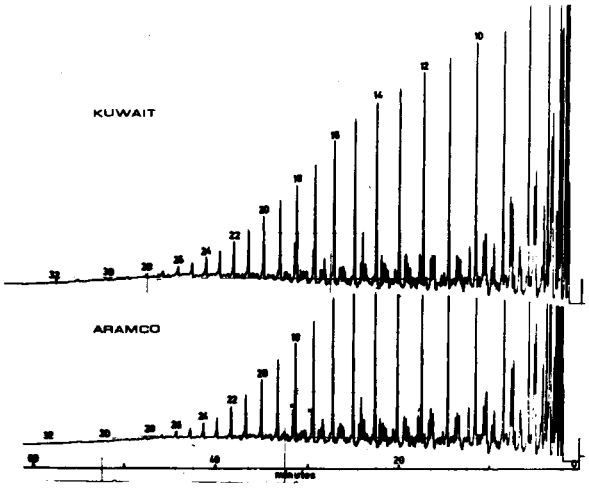

Fig. 4.HR GC profiles (FID) of Kuwait and Aramco crude oils (x : pristane and phytane).

In the case of the identification of oil spillages from tanker washings several additional difficulties arise. In Fig. 6 it can be seen how the fingerprints of the original samples have been modified by the typical sludge peaks (n-C_{25+}) and the lower fractions have been altered (affecting the phytane/pristane ratios) by the long exposure to sea weathering conditions. It is worth to mention here that the classification criteria established in Fig. 2, according to characteristic chemical parameters,

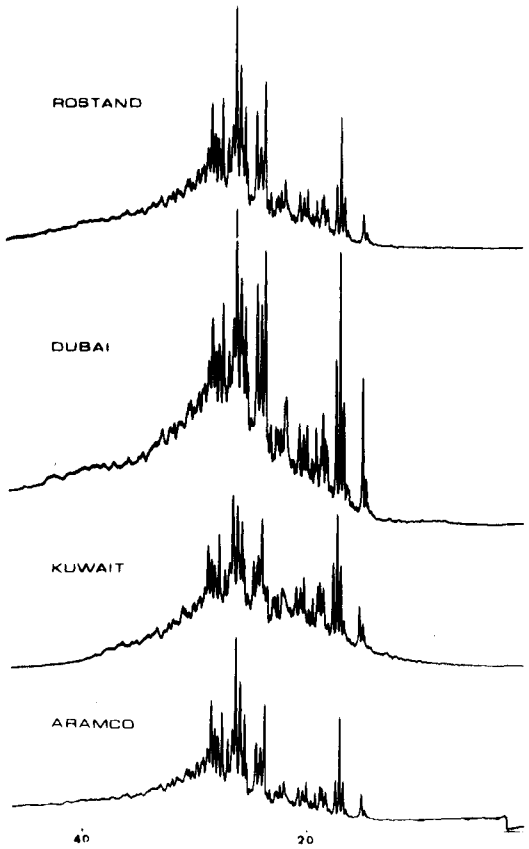

Fig. 5. HR GC profiles (FPD) of Middle East crude oils.

could also give unconclusive results on such samples, owing to their variable enrichment in paraffins during tanker transport or deposition at sea.

A final observation to be made on the applicability of this GC fingerprinting procedure is that the lack of knowledge about the nature of every resolved compound doesn't allow to elucidate the significance of qualitative variations among profiles , thus difficulting to ascertain when the slight differences absorved are attributable to different origins or weathering histories. For these reasons we turned our attention to a more specific sample characterization procedure, which involves the fingerprinting of HC series of geochemical significance (biological markers). The occurrence and distribution of these series, which are included in the unresolved "hump" of the chromatogram, are related to the particular genetic history of the samples.

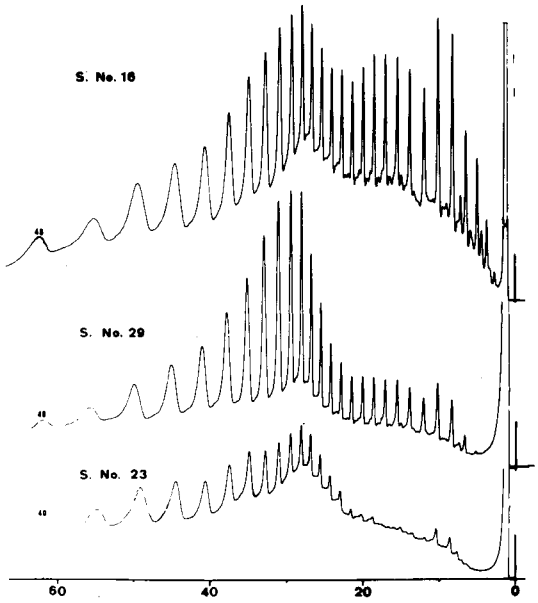

Fig. 6. GC profiles of pelagic tar ball samples collected at the Western Mediterranean. Weathering increases from S.N.16 to No.23.

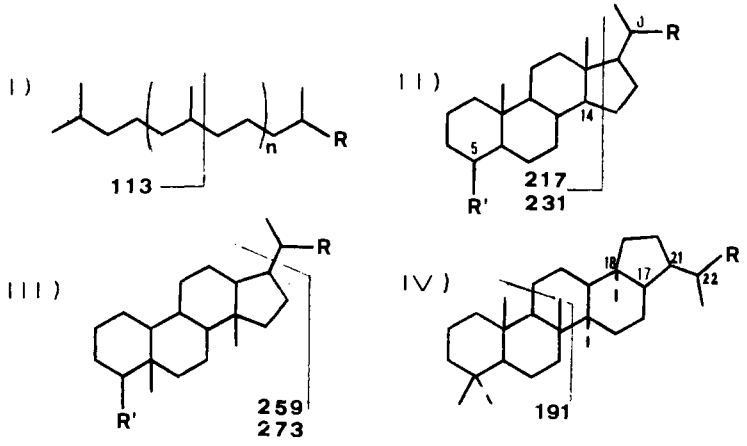

Fig. 7. Petroleum biological markers. I: acyclic isoprenoids. II:steranes. III: rearranged steranes. IV: hopanes. Numbers indicate preferred fragmentation ions in the mass spectra.

Among these series of HC we have the acyclic and polycyclic isoprenoid alkanes drawn in Fig. 7, which seem to be resistant enough to sea weathering (Reed and Kaplan, 1977), thus being suitable for identification purposes. Some recent reports (Rubinstein and others, 1977; Seifert and Moldowan, 1979) have specifically dealt with the biodegradation of these "markers" and the results reported confirm that the profiles are severely modified only when conditions for total degradation of acyclic isoprenoids have prevailed; a rare ocurrence in the marine environment.

Taking into account that these compounds exhibit common ions in their mass spectra (see Fig. 7), the M.F. paterns from computerized GC-MS provide a powerful tool for the determination of distributions of homologous series, affording the relative abundances of individual members and epimeric and ring skeletal isomers. Hence, crude oils quoted in Fig. 2 were characterized by the mass-chromatograms of m/e 183, 191, 217, 231 and 259. Although not all of them provide significant fingerprints for each one of the referred ions, in spite of this, relevant differences were still observed.

Fig. 8 shows a few examples from these crude oils. The most abundant series is that of the triterpanes of the hopane type(IV,m/e 191). This family is formed by a series of C_{27}-C_{35} members. The stereochemistry of the C-17 and C-21 in petroleum and matured HC samples is 17 α (H), 21 β (H) and 17 β (H), 21 α (H) with the 22R + 22S isomers, whilst in the precursor biological materials only the 17β(H), 21α(H) with one diastereomer at position 22 is found. This stereochemical fate has been considered as a definite test for oil pollution monitoring in sediments (Dastillung and Albrecht, 1976). In addition, two C_{27} members are present, the 17α (H) and 18α (H)-trisnorhopanes, their relative abundance being able to differentiate the previously reported Aramco and Kuwait crude oils.

The distribution of the individual members of this series was previously used, after isolation, by Pym and others (1975) to fingerprint M.E. crude oils. However, the advantages of the present MF procedure are obvious as far as the analysis time is concerned, as well as to the new identification possibilities offered by the multiparametric profiles hereing described. Moreover, another identification parameter displayed by the m/e 191 MF is that corresponding to the C_{20}-C_{26} tricyclic diterpanes (Reed, 1977)which elute before the hopanes and appear with an asterisk in Fig. 8.

In the sterane and methylsterane families two series of compounds can be expected: the normal (II, m/e 217, 231) and the rearranged steranes (III, m/e 259, 273), the latter occurring exclusively in petroleum and other geochemically matured samples. Variations in the stereochemistry of carbons 5, 14, 20 and 24 afford a very complex pattern (Ensminger and others, 1978)and give complementary evidence of fossil fuel origin, because the chiral centers are built biosynthetically in only one stereochemical configuration. However, steranes seem to disappear earlier with maturation, hence N.A. crude oils exhibit the common characteristic of having very small concentrations of these HC (Fig. 8).

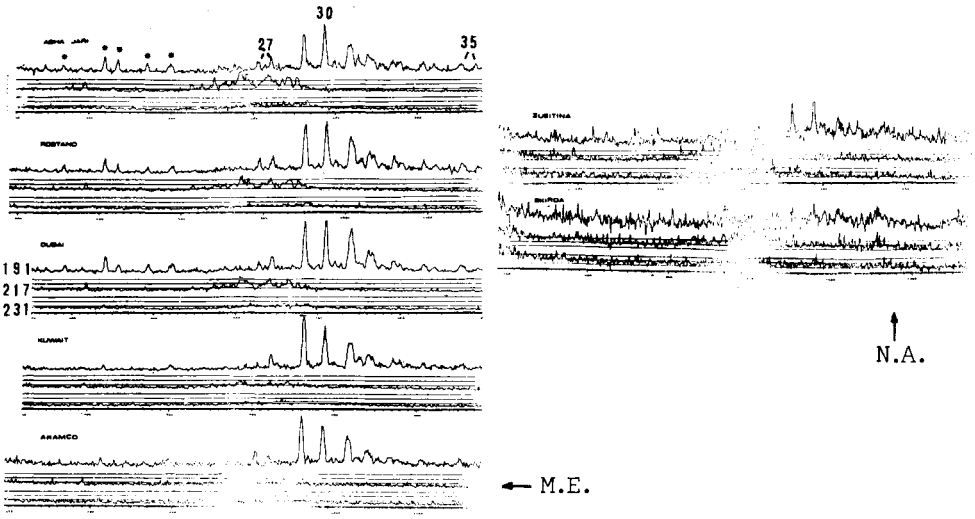

Fig. 8. MF profiles of Middle East (M.E.) and North African (N.A.) crude oils.

Long chain acyclic isoprenoids (C_{25}-C_{40}) (I, m/e 183), although not as usiquitous as the preceding series can be of interest in the identification of specific sources of pollutants. For example, they have been found to be representative of the crude oils produced off-shore in the mediterranean spanish coast; oils that, on the other hand, are difficult to differentiate from some N.A., owing to the similarity of their characterization parameters (Fig. 2).

The ability of this fingerprinting procedure to identify the specific source of long-lived petroleum residues in the open ocean (pelagic tar balls and petroleum particulates) is clearly shown in Fig. 9. The samples range from moderately to highly weathered crude oil sludges, according to their GC profiles, which are similar to those shown in Fig. 6. The chemical and chromatographic approaches for their source identification gave rather dissapointing results. V/Ni ratios were comprised in the ranges given in Fig. 2 for the M.E. and S.U. crude oils; however, the S contents were lower than 1%, probably due to the higher paraffin content of the samples, characteristic of the oil sludges. Finally, phytante pristane rations were affected by the loss of the lower boiling fractions, primarily by evaporation. Evaporation is also resposible for the removal of the most representative part of the GC profiles which is situated in the range of C_{14}-C_{20} n-paraffins (see Fig. 3). Nevertheless, MF profiles, which

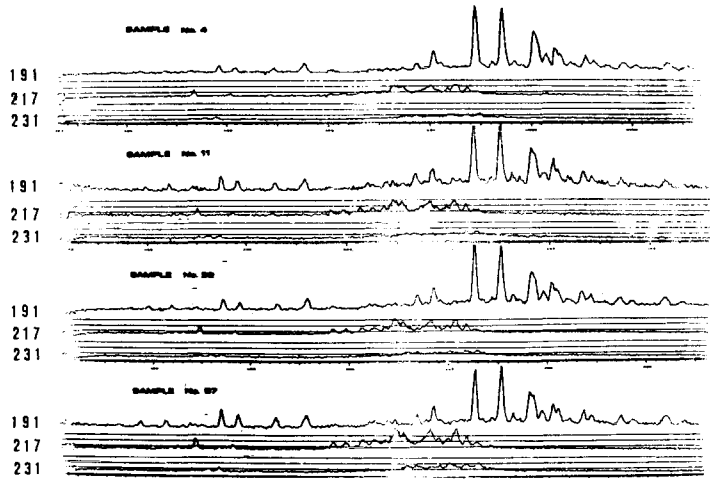

Fig. 9. MF profiles of four pelagic tar balls collected at the Western Mediterranean.

are not modified by any of the referred compositional and weathering factors, afford enough information to permit the correlation of the samples with Iranian crude oils.

In conclusion, it can be pointed out that novel molecular fingerprinting techniques, involving specific geochemical markers, are expected to be more conclusive than gross compositional parameters for determining sources of fossil fuel contamination. HRGC-MS-computer systems are able to furnish the corresponding profiles without complex sample treatments. Multiparametric profiles can be obtained from one run and can be easily stored for further processing. In this respect a general effort is needed in order to complete a fingerprint catalogue of worldwide produced oils.

ACKNOWLEDGMENTS

We are gratefully indebted to the Osborne Company (Pto. de Santa Maria, Cadiz) for financial support under the Award in Environmental Protection. We also thank P. Albrecht and G. Teller for facilities provided for the use of the GC-MS-computer system of the Institut de Chimie (Université de Strasbourg).

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Recent Progress in Polycyclic Aromatic Chemistry and its Significance for Environmental Chemistry

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ABSTRACT

Recent findings from our work on polycyclic aromatic hydrocarbons (PAH) and hetero-aromatic systems are introduced, and their possible significance for environmental chemistry discussed. Subjects include: the localization energy concept in PAH chemistry; relationships between topology, stability and reactivity of PAH; photochemistry of PAH; the use of special photophysical properties of PAH in analysis; high-pressure liquid chromatography of PAH.

Polycyclic aromatic hydrocarbons; topology-reactivity-correlations; luminescence analysis; high-pressure liquid chromatography.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH), polycyclic heteroaromatics and simple derivatives (in particular homologues) of both are found everywhere in the human environment, even though in very low concentrations. For example, they are found in the air, in water, foodstuffs, and so on. The question as to whether the omnipresence of polycyclic organic material (POM) is related solely to civilization or has in addition a biogenous origin is still under discussion. However, arguments pointing to an origin condition solely by civilization are on the increase (Blumer, 1975). The POM contents of fossil materials, and in particular of mineral oil, are not given consideration in this respect.

PAH are always formed when material containing carbon and hydrogen is subjected to temperatures exceeding 700°C, i.e. in pyrolytic processes and with incomplete combustion. In all such cases, a considerable proportion of PAH homologues is formed. These are thermally dealkylated at higher temperatures. If the

starting material also contains hetero-atoms, e.g. oxygen, nitrogen, and sulphur, then hetero-aromatics and their derivatives are formed in addition to PAH. Very reliable ideas on the formation mechanism of PAH in pyrolytic processes have been obtained by studying model systems (Lang, Buffleb and Zander, 1963).

The number of isomeric PAH increases in extraordinarily pronounced degree with increasing molecular size. The ratio of the number of systems known today to the number of the theoretically possible systems drecreases rapidly with increasing number of rings. The inclusion of the variety of possible PAH homologues and of hetero-aromatics in the discussion indicate that even optimum analytic methods in keeping with the present state of the art can give no more than an approximate impression of the actual complexity of real samples from the environment. Lao and colleagues (1972) quantitatively detected 150 different PAH in city air using the capillary gaschromatography/mass spectroscopy combination. Grimmer, Böhnke and Glaser (1977) characerized with the GC/MS combination 150 components of vehicle exhaust gas as PAH. It proved possible to positively identify 73 of these by comparison with test substances.

The worldwide interest of environmental chemists in PAH is based on the suspicion of a cancer risk for humans through certain PAH, a suspicion stimulated by mutagenicity screening tests, animal experiments, and epidemiological investigations. The majority of scientists nowadays accept purely biochemical models of carcinogenesis through chemical compounds, but some very interesting investigations (Popp, 1976) also permit the interpretation of carcinogenesis on a solely biophysical basis, particularly in the case of PAH. Within the framework of the models first mentioned, a fundamental distinction is made between chemical carcinogens having an indirect and a direct effect. The indirect-acting carcinogens require metabolic activation, whereas this is not the case with the direct-acting carcinogens. The active form of the carcinogen (ultimate carcinogen) is always an electrophile, which forms bonds with negatively-charged, nucleophilic cellular biopolymers, and in particular DNA. PAH as definite nucleophiles require metabolic activation to ultimate carcinogens with arene oxide groups (Daly, Jerina and Witkop, 1972).

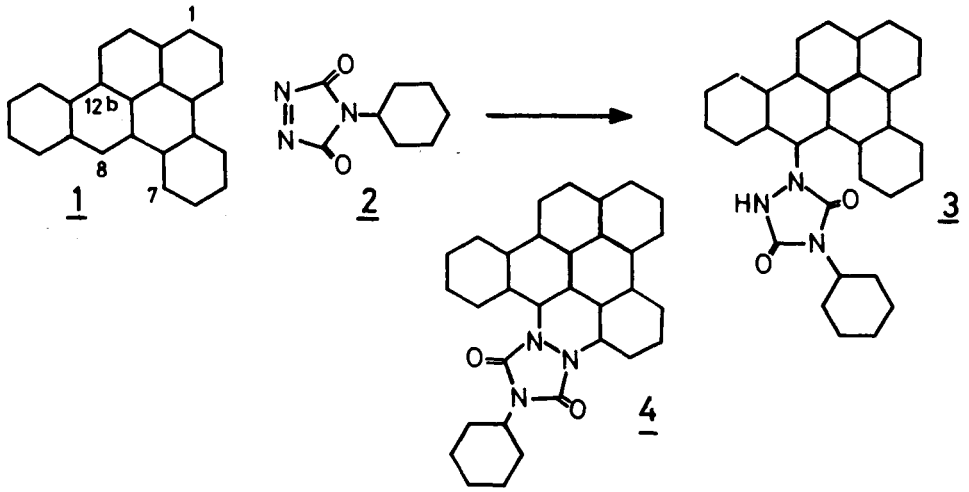
Independent of carcinogenesis model in question, a deeper understanding of the physical and chemical characteristics of a group of carcinogens, e.g. of PAH, is of great value, and in particular an understanding of the relationships between the characeristics and the topology of the systems. Concepts which permit deductions to be made on the stability of compounds under environmental conditions from the topology of systems are useful to the environmental chemist. The organic chemist and the quantum chemist can make valuable contributions in this respect. A deeper understanding of the characteristics of a class of compounds often yields indicators for the development of new methods of analysis, and there can be no doubt that progress in environmental chemistry is linked in high degree with progress in analytical chemistry.

The following reports on observations, methods, and concepts made and developed by us over the past few years - partly in cooperation with other groups - in the sector of PAH. Even though only a limited number of our objectives were directly connected with environmental chemistry, it is possible that many aspects of our findings are of significance for the environmental chemist.

THE LOCALIZATION ENERGY CONCEPT IN PAH CHEMISTRY

In the field of PAH, many addition reactions are known, e.g.those of the Diels-Alder type. With other typical PAH reactions, e.g. with electrophilic substitution, an addition step determines the rate of reaction. For addition reactions at aromatic bonds, Whelands concept of localization energy has proved useful. Localization energy L_u is the energy - in the following, given in units of resonance integral ß - required to isolate a π-electron at the centre u from the remaining π-system. The smaller L_u, the greater the relative reaction rate constant of the addition step under consideration. Of the various known reactivity indices, which are a measure for the localization energy, Dewar's reactivity number Nu (Dewar, 1975) has two advantages: the N_u values correlate extremely well with experimental data, and the N_u values can also be calculated extremely easily for systems having a very big number of centres. One disadvantage of Dewar's method lies in the fact that in its simple form it is applicable only to even-alternant π-electron systems.

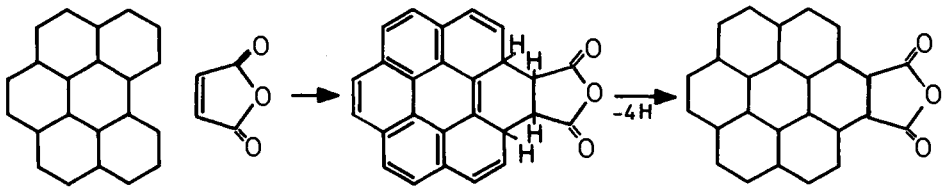
In the following example, Dewar localization energies are employed in interpreting the reactive behaviour ofaPAH, dibenzo[a,e]pyrene (1). In 1, centre 8 of all centres capable of substitution has the lowest N_u value (1.32). Typical electrophilic substitution reactions such as bromination and Friedel-Crafts acylation do not take place in the 8-position, however, but in the 1-position with the next higher N_u value (1.53) (Lang and Zander, 1965). The



fact that the reactions do not take place in the 8-position is very probably due to a steric hindrance. Surprisingly, however, the reaction of 1 with 4-phenyl-1,2,4-triazoline-3,5-dione (2) yields substitution product 3 (Zander, 1975). 2 is a very strong dienophile, and it is thus very probable that initially a Diels-Alder adduct is formed with 1, this adduct finally being rearranged to yield 3.

Neglecting for the moment steric aspects, the virtually identical 8,12b- or 7,8-bislocalization energies (3.24 and 3.29 β^{-1}) yield no indication as to wether the attack takes place at the exo-cisoid or the endo-cisoid C_4 grouping. Steric considerations point to the 8,12b-addition, as does the observation that only 3 is obtained even with a heavy 2 surplus. In the case of an addition in the 7- and 8-position, aromatization of the Diels-Alder adduct to 4 would have to be expected, surplus triazolin derivative acting as the dehydrogenating agent, as has been observed in other cases (Zander, 1975).

Within each reaction type, there is a linear correlation between the logarithms of the relative reaction rate constants and the Dewar localization energies. PAH of the perylene and benzo[ghi]-perylene series react with maleic anhydride in the presence of a dehydrogenating agent to form fully aromatic dicarboxylic acid anhydrides (Clar and Zander, 1957).



The primary formation of the Diels-Alder adduct is the rate-determining step in this "benzogenic Diels-Alder reaction" (Zander, 1969a), which is possible to isolate in some cases (in the absence of a dehydrogenating agent) (Clar and Zander, 1958; Clar, 1972). Fig. 1 shows the logarithms of the relative reaction rate constants of the benzogenic Diels-Alder reaction for several PAH of the perylene and benzo[ghi]perylene series plotted above the bislocalization energies (x β^{-1}) of the reacting centres. The slope of the straight line is proportional to the resonance integral β, which assumes different values for different reaction types and in the linear correlation between reaction rate constant and localization energies equates formally with the reaction constant ρ in the Hammett function.

The examples discussed above show that the Dewar localization energies - which are extremely easy to calculate - permit very good qualitative and quantitative interpretations and predictions of the reactive behaviour of PAH.

In principle, the relevant N_u value can be calculated in respect of each carbon centre of a PAH. Accordingly, there is a N_u

pattern for each PAH. Initially, no relationships are to be recognized between the N_u pattern and the topology of the systems. They are revealed, however, if Clar's π-electron model of PAH (Clar and Zander, 1958; Clar, 1972) and Polansky's pars-orbital concept (Polansky and Derflinger, 1967) are included in the deliberation.

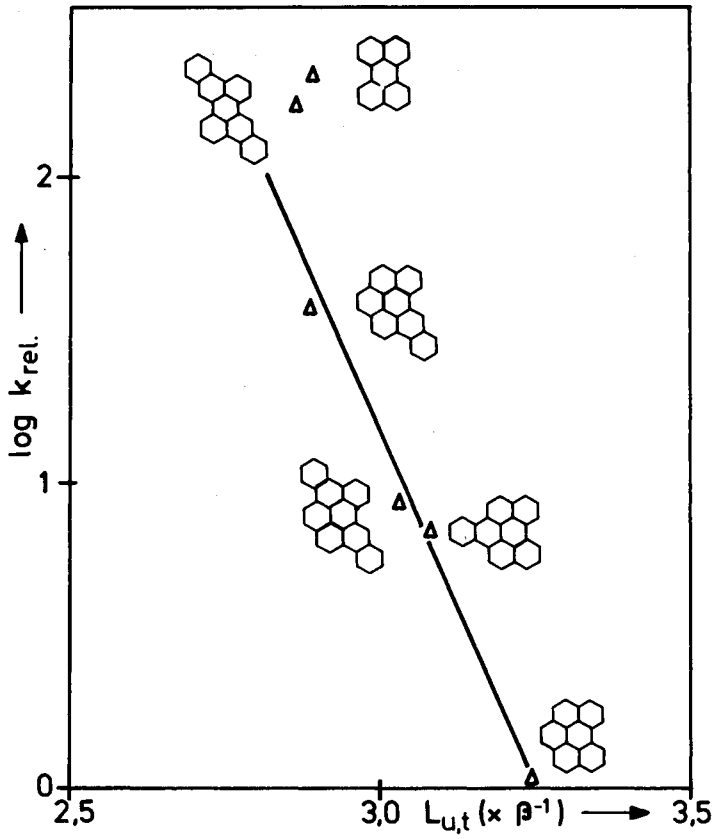


Fig. 1: Relationship between log k_{rel} of the benzogenic Diels-Alder reaction and Dewar bislocalization energies (x ß-1) of the reacting centres

RELATIONSHIPS BETWEEN TOPOLOGY, STABILITY, AND REACTIVITY OF PAH

The resonance energy per π-electron ("specific resonance energy") is a suitable factor for representing the correlations between topology and stability of PAH. In Fig. 2, the specific resonance energy of benzene, naphthalene, anthracene, and tetracene is plotted above the total number of π-electrones of these

systems. The diagram shows the usual stability decrease with increasing annellation.

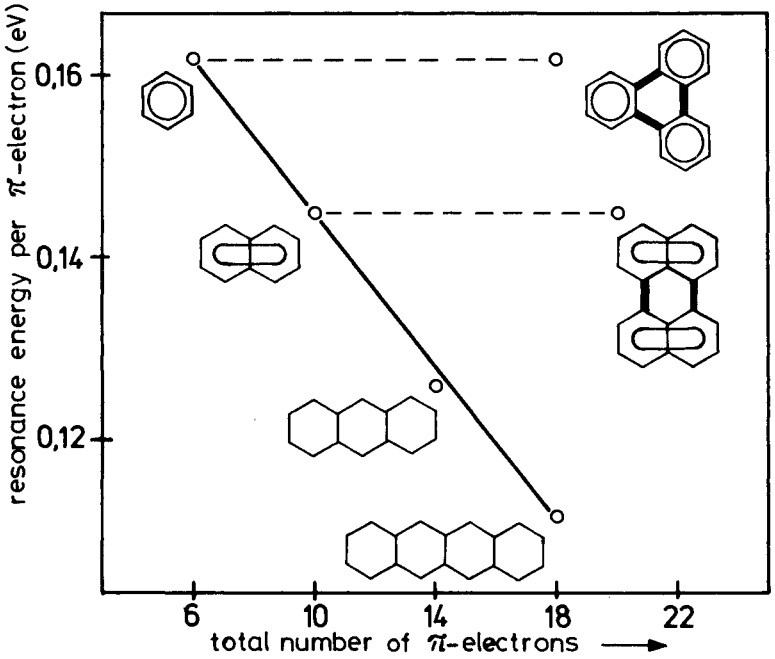
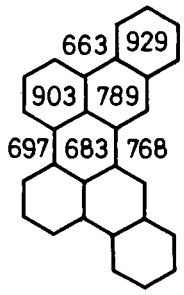


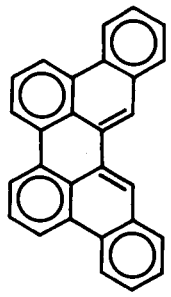
Fig. 2: Resonance energy per π-electron of several PAH as a factor of the total number of π-electrons

It is even more remarkable that triphenylene has the same specific resonance energy as benzene, and perylene the same as naphthalene. On a purely formal basis, it follows that triphenylene can be considered as constituting of three localized benzene units, and perylene of two localized naphthalene units. This is shown in the formulae, in which the thicker lines denote "quasi-single bonds". The existence of localized π-electron ranges in PAH was first postulated by Clar (Clar and Zander, 1958). Clar (1972) developed this concept on the basis of numerous experimental results to build up a comprehensive structural-chemical model of PAH.

Polansky and Derflinger (1967) developed the quantum chemical verification of this model by using as bases in the Hückel approximation for the linear combination the HMO of benzene and other partial structures recognizeable in PAH (butadienoids, allyloids, and ethylenoids) instead of the usual atom orbitals. In this way, they obtained the "character orders" of these partial structures; according to the analogy principle, the "bonding properties" of a partial structure equate the more with those of the reference compound (benzene, butadiene, etc.) the greater the character order of the partial structure in question. The charac-

ter order measures the contribution of the bonding orbitals of a partial structure to the bonding orbitals of the entire molecule. By way of example, the formula includes the relevant character orders of dibenzo[b,n]perylene.

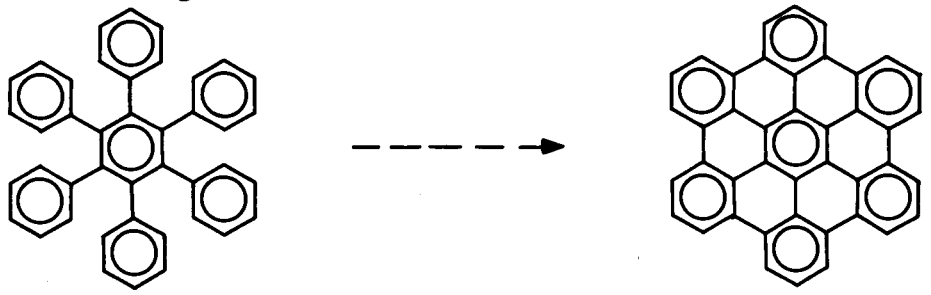


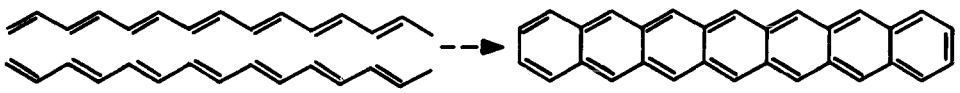


The figures in the hexagons are the benzoid character orders, the remaining figures the butadienoid character orders of the cisoid C_4 partial structures. The formula on the right gives the qualitative bonding properties in dibenzo[b,n]perylene.

The Polansky character orders correlate well with the experimental data such as NMR coupling constants, magnetic susceptibilities, half-wave potentials, and reaction rates (Fratev, Polansky, and Zander, 1975).

The model indicates that in a series of isomeric PAH stability increases with the number of the benzoid partial systems. In fact, PAH such as triphenylene (see Fig. 2), which can also be thought of as purely benzoid partial systems linked by"quasi single bonds", are the most stable PAH known (Zander, 1960). Such "all-benzoid" PAH can be imagined as having been formed by innermolecular ring closures of polyphenyls.



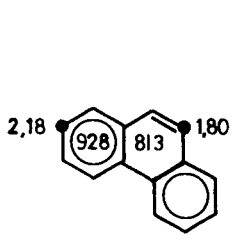


The acenes are found at the other end of the stability scale of PAH. An infinitely long acene is formally created by linking two polyene chains; it could be described as being an "all-alkenoid". Heptacene, with 7 linear annellated rings, is the most high-mole-

cular representative of this structural principle known to date, and is an extremely unstable PAH. Heptacene could be designated as being a "quasi all-alkenoid". Between these two boundary cases - the all-benzoid and the quasi all-alkenoid structural principle - lie numerous different structural principles of differing stability; the simple relationship between the number of benzoid partial systems and stability is to be noted in all cases.

THE CORRESPONDENCE BETWEEN N_u PATTERN AND CHARACTEROGRAMS OF PAH

The organic chemist would intuitively expect that centres belonging to partial structures of high benzoid character order in a PAH are distinguished by high N_u values (high localization energies), and that conversely centres belonging to partial structures of low benzoid character order have small Nu values (low localization energies). This is in fact the case within certain limits.



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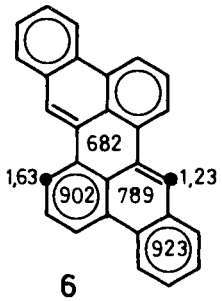

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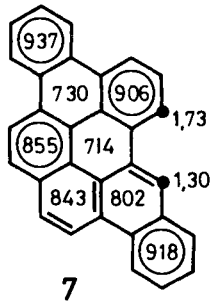

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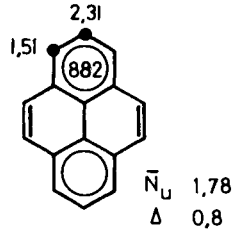
Here are some examples : of the topologically equivalent centres of phenanthrene and dibenzo[b,k]perylene marked in formulae 5 and 6, the centre which in each case belongs to the ring with the lower benzoid character order has the lower N_u value, and vice versa. The same applies to the topologically equivalent centres of tribenzoperylene marked in 6 and in formula 7. Also, on transition from 6 to 7, both the appropriate benzoid character orders and the N_u values increase.



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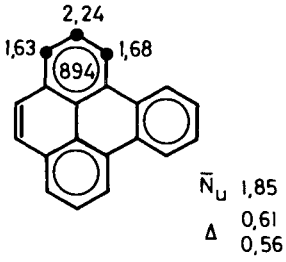

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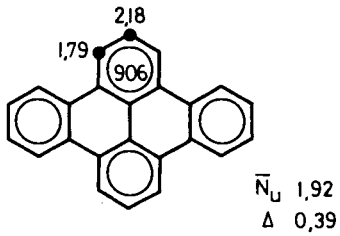


10

If in the series pyrene (8), benzo[e]pyrene (9), dibenzo[e,l]pyrene (10), benzoid character orders and N_u values of topologically equivalent rings and centres, are compared it will be seen that

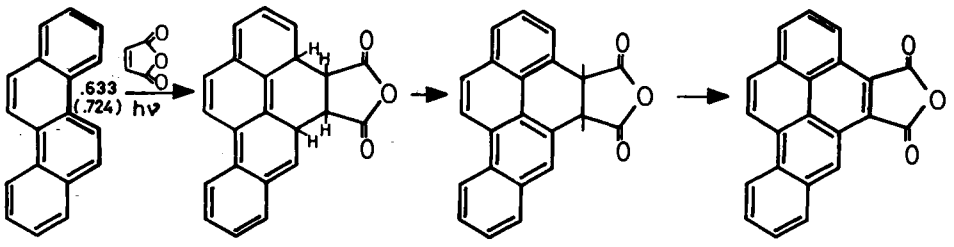
with increasing benzoid character order the arithmetic mean $\bar{N}_u$ of the N_u values of the secondary carbon centres of the ring under consideration increases (a very good linear correlation existing between benzoid character order and $\bar{N}_u$), and also that the differences between the individual N_u values forming $\bar{N}_u$ decrease. All in all, the character orders and the N_u values indicate that the ring under consideration in the series 8, 9, 10 becomes more "benzene similar". This agrees with the experimentally demonstrated chemical behaviour of the three compared PAH. Numerous other examples of this type prove that a correspondence exists between N_u pattern and benzoid character orders of PAH (partial characterograms) (Zander, 1978).

CHARACTER ORDERS IN THE EXCITED STATE, AND PHOTOCHEMISTRY OF PAH

The long-established observation that the concentration of benzo-[a]pyrene and other PAH in city air is lower during the summer months than in the winter months can only in part be explained by the absence of emissions from domestic heating systems during the summer months; a further reason is to be found in the more pronounced photochemical degradation of PAH during the summer months The photochemical (and photophysical) behaviour of PAH is also of interest in connection with investigations into carcinogenesis (Cavelieri and Calvin, 1971).

Polansky (1974) demonstrated how within the pars-orbital concept the character orders of partial structures of electronically excited molecules can be derived. Here, the charcter orders are so defined that they describe the analogy between the reference compound (e.g.: butadiene) in the ground state and the relevant (e.g.: butadienoids) partial structure in the electronically excited molecule.

Formula 11 shows for chrysene the butadienoid character orders in the ground state and in the electronically excited state (values in brackets).



11 12 13 14

In agreement with the character orders, chrysene reacts under photochemical conditions with maleic anhydride in the sense of a Diels-Alder reaction, whereas in the relevant thermal experiment no reaction is to be noted (Karpf, Polansky and Zander, 1978). In the presence of atmospheric oxygen (which acts as a dehydro-

genating agent), compound 14 is formed in the photochemical experiment, whereas compound 13 is formed in the absence of air. A plausible explanation would be that the Diels-Alder adduct 12 to be expected as the primary product of the reaction is very unstable as a result of its structure and easily forms 13 (e.g. dehydrogenation and/or disproportionation).

Further experimental examples indicate that the pars-orbital concept can be of use in the interpretation and prediction of the photochemical behaviour of PAH, too, and is thus also of significance for the environmental chemist interested in PAH.

THE UTILIZATION OF SPECIFIC PHOTOPHYSICAL PROPERTIES OF PAH IN ANALYSIS

In view of their sensitivity and selectivity, luminescence spectroscopic methods (fluorimetry and phosphorimetry) are of use in PAH analysis. Recently, techniques have gained significance in fluorimetry and phosphorimetry which permit - through the addition of suitable compounds to the solution - the selective intensification of the luminescence of individual components of mixtures (enhancophosphorimetry; Zander, 1969 b) and also the selective quenching of these (quenchofluorimetry; Sawicki, Stanley and Elbert, 1964; Zander, 1973). In some cases, these techniques can replace the usually time-consuming chromatographic separation methods; in combination with thin-layer chromatography, they enhance the analytical information yielded.

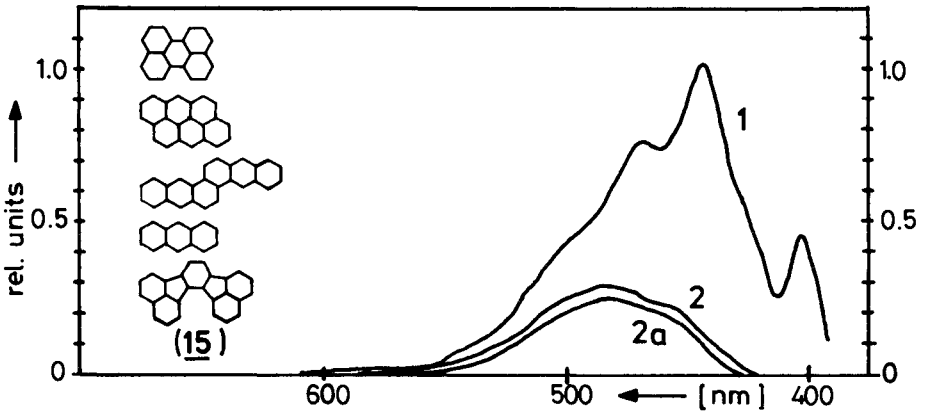


Fig. 3: Fluorescence spectra (acetonitrile, room temperature, excitation wavelength 383 nm). Curve 1: spectrum of mixture with composition shown on left; Curve 2: spectrum of mixture in presence of 50 % by volume nitromethane; Curve 2a: spectrum of acenaphtho[1.2-j]fluoranthene (15)

According to an observation made by Sawicki, Stanley and Elbert (1964), the fluorescence of non-alternant PAH (systems containing the fluoranthene skeleton) is not quenched by nitromethane, in contrast to that of alternant PAH. Sawicki used this observation mainly to identify PAH of the fluoranthene series on thin-layer chromatogrammes of complex PAH mixtures. Selective fluorescence quenching with nitromethane is also a useful method in the analysis of PAH mixtures without being linked with a chromatographic separation process; its application is particularly expedient in cases where the PAH in the mixture have substantially similar fluorescence and absorption spectra, so that the success of a discrimination through selective excitation must remain limited. Fig. 3 gives an example of this method: Curve 1 is the fluorescence spectrum of a mixture of 4 alternant and one non-alternant PAH in acetone nitrile at room temperature. The fluorescence spectrum of the mixture following the addition of 50 % by volume nitromethane to the solution is shown in Curve 2. As a comparison with the spectrum of the pure PAH in Curve 2a shows, it now comprises only the spectrum of the non-alternant PAH (Breymann and colleagues, 1978).

One result yielded by detailed photophysical investigations into the internal mechanism of fluorescence quenching by nitromethane and other electron acceptors is that with electron donors - for example 1,2,4-trimethoxybenzene - as quenchers, the fluorescence of non-alternant PAH is substantially more easily quenched than that of alternant PAH. Fig. 4 shows an example of the analytic use of this inverse quench effect: Curve 1 is the fluorescence spectrum of a mixture of 4 non-alternant PAH and one alternant PAH.

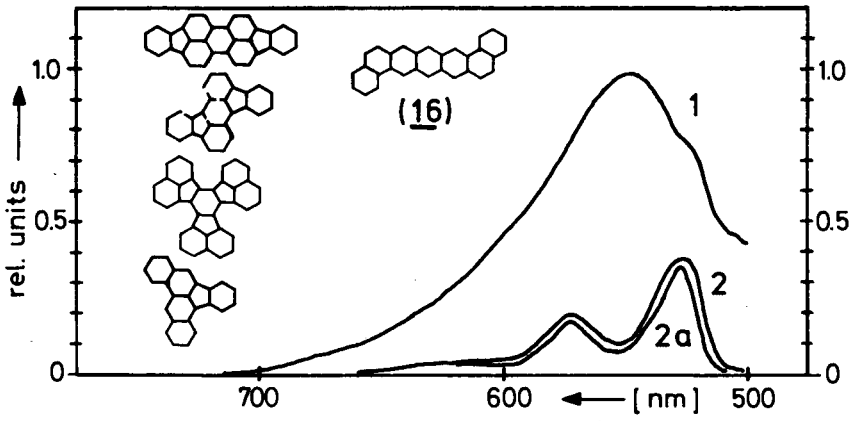


Fig. 4: Fluorescence spectra (acetonitrile, room temperature, excitation wavelength 480 nm). Curve 1: spectrum of the mixture with the composition shown on the left; Curve 2: spectrum of the mixture in the presence of 40 % by volume 1,2,4-trimethoxybenzene; Curve 2a: spectrum of dibenzo[a,l]pentacene (16)

Following the addition of 40 % by volume 1,2,4-trimethoxybenzene to the solution, spectrum 2 is obtained; this completely agrees with the spectrum of the pure alternant PAH (Curve 2a) (Breymann and colleagues, 1978).

Complementary quenchofluorimetry with electron acceptors and electron donors permits the selective proving of alternant and non-alternant PAH in PAH mixtures. In many cases, quantitative analysis are also possible. In addition to PAH analysis, the method is also useful in the structure determination of PAH (Blümer, Gundermann and Zander, 1976).

THE APPLICATION OF HIGH-PRESSURE LIQUID CHROMATOGRAPHY IN PAH ANALYSIS

The bulk of the numerous publications over the past few years on the application of high-pressure liquid chromatography (HPLC) in PAH analysis (for a review, see: Thoms and Zander, 1977) relate to PAH mixtures which can also be analyzed by gas chromatography. The fundamental limit to the use of gas chromatography is set by the required volatility of the substances; according to our experience, PAH with mol masses of up to approx. 350 can be gas-chromatographically detected. The solubility of the substances being examined is the sole fundamental condition for the employment of HPLC. Examples exist for the HPLC detection of PAH with mol masses of up to approx. 600 (Thoms and Zander, 1976; Blümer and Zander, 1977). Compared with HPLC, gas chromatography is much easier to apply in quantitative analyses and has an excellent separation capacity - e.g. with capillary columns. Accordingly, gas chromatography should always be used where this is possible as regards the volatility of the PAH and their thermal stability. HPLC is expediently used in the range of very high molecular and/or thermally very unstable PAH, which according to present findings can not be analyzed by gas chromatography.

Silica gel modified with nitrophenyl groups as a stationary HPLC phase has the specific property of retaining for very long periods aza-aromatics, carbazoles, and other nitrogen compounds; this is probably due to charge-transfer interactions. Carbocyclic aromatics and aza-aromatics up to a C number of 28, equalling a molecular weight of approx. 350, can be group-specifically separated at this phase. Separation within the groups is excellent (Blümer and Zander, 1977). This method should prove to be of use for the analysis of real samples from the environment.

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Determination of Particulate Organic Matter in Environmental Samples by Gas Chromatography-Mass Spectrometry and High Pressure Liquid Chromatography

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ABSTRACT

Polynuclear aromatic hydrocarbons (PAH) in environmental samples must be measured at low concentration levels. Gas chromatography-mass spectrometry (GC/MS) coupled with a data processor provides a method that is both sensitive and selective. The practical usefulness of the system has been well demonstrated for the trace analysis of PAH. The preliminary sample treatment prior to the GC/MS analysis involves various partitioning sequences such as collection, extraction, concentration, and separation. These steps are generally complex, unit process time is excessive, and must be handled with care to prevent losses of PAH and sample contaminations. With the recent development of high pressure liquid chromatography (HPLC) technique, a new analytical approach for PAH determination has been developed. In this work the application of GC/MS and HPLC to the identification and measurement of complex PAH mixtures will be discussed. Results will be compared.

KEYWORDS PAH analysis by GC/MS and HPLC

INTRODUCTION

In the preceding two decades a considerable volume of information has been published throughout the world on the particulate organic matter (POM). The POM also known as polynuclear aromatic hydrocarbons (PAH or PNA) are among the most widespread contaminants of the environment, generally attributable to incomplete combustion and pyrolysis of carbonaceous fuels or other organic materials. PAH are present in nature from sources such as asbestos, tobacco tar and in ambient air formed by secondary physico-chemical reactions, and the number of compounds and their concentrations are increased by emissions from industrial activities (IARC, 1976; NAS, 1972).

Concern over the prevalence of PAH has developed from evidence that a large number of these compounds are known to be carcinogenic or co-carcinogenic resulting from oxidative reactions in the body. Statistical studies have attempted to establish a correlation between concentration of PAH and cancer mortality rates of various population groups (IARC). In addition studies have related benzo[a]pyrene(BaP) in polluted urban air to lung cancer A number of urban atmospheric studies have been completed and concentrations of BaP, and other biologically active compounds have been reported(IARC).

Trace analysis of PAH even today remains in a developmental stage and inspite of significant advances in measurement techniques, current analytical methods are not absolute nor standardized. The main steps in PAH analysis include collection, storage, transportation, extraction, concentration,identification and quantification. The first three steps are often ignored and become a source of error. The situation is often beyond the control of bench-work analysts. The samples collected may be contaminated or without consideration of the physico-chemical properties of the PAH, which results in meaningless measurments. The choice of a collection system must be a primary concern (Pupp) in air sampling. Few changes have been made to the methodology of collection while the analytical techniques for pollutant assessment in air are continually being evaluated and upgraded. Traditionally, airborne particulates have been sampled by drawing a known volume of contaminated air through a filtration medium using a high volume sampler. The fact that simple filtration sampling has been universally applied without re-evaluation has serious implications when it is realized that many PAHs have high vapor pressures and will either pass through the filter medium without being entrained or will be re-evaporated from the particulates collected by the filter. (Pupp, 1974)

The collection efficiency of the sampler must be established before any consideration of PAH analysis. The failure to collect a concentration equal to the equilibrium vapor concentration(EVC) and subsequent sublimation from the filter are two causes of collection inefficiency when using particulate filtration (Pupp).Losses due to sublimation during storage, transportation or sample concentration process are also proportional to the EVC.(Pupp) In the case of water, effluent and sediment samples, PAH collected in the container are susceptible to a number of processes including photo or thermal decompositions, wall adsorption, microbial and chemical reactions. For aqueous samples the choice of sample containers and storage conditions become of vital importance(Hoffman, 1978).

Organic solvents have been traditionally used as extracting agents, and more recently inert gasses have seen limited applications. Several studies have considered the effects of the solvent mixture selection for classes and concentration ranges of PAH extracted from environmental samples. Air samples collected on the filter media are usually extracted by soxhlet or ultrasonic methods. Generally soxhlet extraction procedures require at least eight hours whereas the ultrasonic process can be completed within an hour. Sample decomposition may prove a limitation for the use of the ultrasonic extraction process. However, the slower speed, lower extraction efficiency and the possibility of changes in biological activities are the main disadvantages of soxhlet techniques. Cyclohexane and benzene have been the common extracting solvents. Choice of solvent is defined by the extraction for the particular sample matrix, compatibility with subsequent clean-up procedures and the analytical methods adapted. Pre-concentration columns using varieties of polymeric resins have been used to extract PAH from water or industrial effluents(Kaiser, 1974; Zlatkis, 1974).

Often extraction procedures are non-selective and will extract a broad spectrum of organic compounds from the environmental samples. Preliminary separation and isolation steps become necessary. Chromatography in one form or another such as column (CC), liquid-liquid partition and thin layer(TLC) are used to produce specific class separations. The separation procedures for PAH are selected to isolate these compounds from the aliphatic and heterocyclic components of the "neutral" extract (Hoffman, 1978).

PAH separation by CC may be accomplished on alumina, silicagel or Florisil of uniform particle size. The separation of PAH concentrates into specific subfractions has been successfully achieved on the lipophilic gel LH-20 Procedures based on paper chromatography (PC) have only limited practical application. The TLC and HPLC have gained increasing popularity as replacements for the time-consuming and intensive manual operations of traditional CC or PC. However, the TLC technique is still in the development stage and is seldom applied to routine PAH analysis. New development in high resolution column packings provide HPLC the advantages of speed, resolution and solvent economy. A number of difficult separations can be accomplished by the careful selection of solvent polarity blends, pressure programming and recycling the sample which contains specific PAH components.

A concentration procedure is often required prior to any analytical finish, since the final extract of the separated sample may be diluted by the solvent. Great care must be taken during the volumetric reduction of the solvent to prevent the loss of volatile PAH components. A rotary vacuum apparatus or a Kuderna-Danish concentrator, etc is satisfactory for concentration of samples from several hundred ml to a few ml or less (Hertz, 1978).

Any impurities present in the solvent or adsorbents used for the column separation are subject to a several hundred-fold concentration by the evaporated step. Blank determinations are necessary for all solvents and other materials used during the sample preparation.

A wide variety of analytical schemes have been developed for the identification and quantification of PAH in an environmental sample. Ultraviolet (UV) absorption and optical fluorescence spectrophotometric methods have been used in PAH analysis for more than twenty years, but the techniques require long detailed clean-up procedures and have limited application to complex mixture analysis. Infrared spectrophotometry (IR) and nuclear magnetic resonance spectrometry (NMR) have been used to provide structural elucidation of some PAH. Their sensitivity limitations, however, make them unsuitable for trace determinations. Although significant progress has been made in other promising techniques such as electrochemical systems, plasma chromatography and room temperature phosphorescence, the applications of these techniques to PAH are preliminary and instrumentation based on these principles are either not commercially available or unsuitable for routine PAH analysis (Gammage, 1977).

Combined gas chromatography/mass spectrometry(GC/MS) or HPLC/MS are currently the most powerful techniques for the identification and measurement of trace PAH in environmental samples. GC/MS provides the analyst with the opportunity of positive PAH identification and has sufficient sensitivity for trace analysis(Lao, 1977).Developments in chromatographic packings and columns, particularly the high-performance glass capillary, have greatly enhanced the applicability of the GC system and eliminated many of the resolution problems present in packed column studies. Coupling a MS to a GC as detector provides a versatile detecting system with high sensitivity and selectivity. A detailed description of the system has been reported in the literature, (Fenselau, 1977).

Recently, automated HPLC systems have been applied to the PAH analysis in a variety of encironmental samples (Lao, 1978). Variable wavelength UV and absorption and optical fluorescence detectors have been used separately or in tandem for monitoring the column eluate. A continuous HPLC/MS system is now commercially available (McFadden, 1977) and it can be modified to handle very

TABLE 1 List of PAH Standards

21 PAH System

Name	Abbreviation	Name	Abbreviation
1. Fluorene	FL	12. Chrysene	Chry
2. 9-Fluorenone	Flone	13. Naphthacene	N
3. Phenanthrene	PA	14. Benzo[k]fluoranthene	BKF
4. Anthracene	AN	15. Benzo[e]pyrene	BeP
5. Acridine	AC	16. Benzo[a]pyrene	BaP
6. Carbazole	Car	17. Perylene	Pery
7. Fluoranthene	FA	18. 3-Methylcholanthrene	MC
8. Pyrene	Py	19. Dibenzo[ah]anthracene	DBahA
9. 11-H-Benzo[a] fluorene	BaF1	20. Benzo[ghi]perylene	BghiP
10. 11-H-Benzo[b] fluorene	BbF1	21. 7-H-Dibenzo[cg]carbazole	DBchC
11. Benzo[a]anthracene	BaA		

13 PAH System

1. Fluoranthene		8. Benzo[a]pyrene	
2. Pyrene		9. Benzo[e]pyrene	
3. Triphenylene	Trip	10. Benzo[k]fluoranthene	
4. Benzo[ghi] Fluoranthene	BghiF	11. Perylene	
5. Chrysene		12. Benzo[ghi]perylene	
6. Benzo[a] anthracene		13. Coronene	Cor
7. Benzo[b] fluoranthene	BbF		

concentrated samples on small volume columns.

A major difficulty in PAH analysis was the lack of individual compounds of defined purity for use as internal standards for calibration and identification. The recent increased availability of the standard reference materials has greatly facilitated the characterization of environmental samples. It is important to note that blends of PAH compounds originating from coal tar or petroleum products will contain isomeric impurities or alkylated derivatives of the parent compounds. For all reference purposes, the qualitative purity of PAH must be assigned and defined. In this work both GC/MS and HPLC systems were used for the investigation of standard PAH mixtures and industrial samples. The performance of GC columns were examined under a series of nominal operational parameters. Results obtained from different analytical systems are studied and discussed.

EXPERIMENTAL PROCEDURE

Reagents

PAH standards were obtained from the laboratory collection and were prepared by various suppliers. Table 1 gives the names of these compounds and abbreviations used in the text. The mixtures used for evaluation were described in a previous study (Lao, 1978). All these compounds have been isolated from environmental samples. The procedures for determining the GC and HPLC parameters have been reported (Lao, 1978, 1979). Standards were weighed by microbalance and made up volumetrically in spectrograde cyclohexane. The

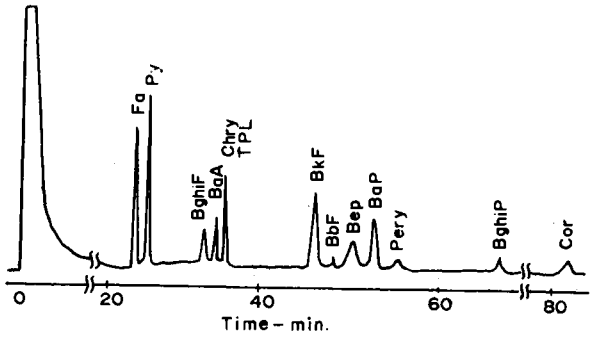

Fig. 1. Gas chromatogram of 13 PAH standards on Dexsil-300 glass capillary column.

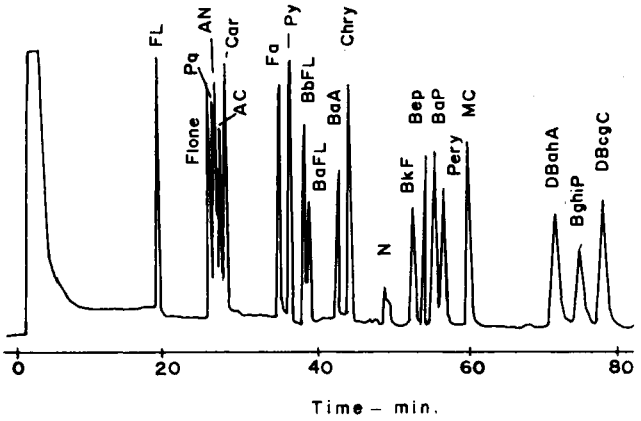

Fig. 2. Gas chromatogram of 21 PAH standards on Dexsil-300 glass capillary column.

acetonitrile for HPLC was spectrograde quality and the water was purified using a Millipore Super Q system followed by vacuum filtration and degasing.

Instrumentation

Two parallel GC systems were used in addition to the HPLC. Operating parameters and instrumental data are summarized in Table 2. Qualitative confirmation of chromatographic peaks was provided by a Finnigan 1015D GC-MS/model 6000 data processor system. All apparata and reagents were evaluated by GC-flame ionization detector (FID) to verify the absence of PAH residue contamination. Details of the instrumental calibrations, interpretation procedures and column specifications have been documented (Lao, 1973, 1978, 1979).

Samples

The first sample investigated in this study was stack emission and was obtain-

TABLE 2 Instrumental Data

GC	Perkin-Elmer 990	Perkin-Elmer Sigma I
Detector	FID	FID
Detector temperature	300°C	350°C
Column	packed, WCOT, Dexsil-300	Glass capillary
Sample volume	0.2 - 3 µl	1 - 5 µl
Sample injector temp.	325°C	325°C
Column Specifications		
	Packed: 12 feet long, 0.125" I.D. 6% Dexsil-300 on 80/100 mesh Chromosorb W (HP)	Dexsil-300 50 meter long, 0.25 mm I.D.
	WCOT: 150 ft long 0.02" I. D. stainless steel preheated and stabilized at 200 C	SE-52: 30 meter long, o.25 mm I.D.
Carrier gas flow	Packed: N 40 ml/min WCOT: N 3.2 ml/min	N : o.8 ml/min
Split ratio	15:1 for WCOT	150:1
Initial temperature	175°C	160°C
Programmed temperature	4 C/min	4 C/min
Final temperature	280°C	270°C
Recorder attenuation	from 4 to 16	4

GC-MS	HPLC
Instrument: Finnigan Model 1015D: Operational conditions are the same as described in Ref. Lao, 1973.	Instrument: Waters Associates Model ALC/GPC system: Operational conditions are the same as described in Ref. Lao, 1978.

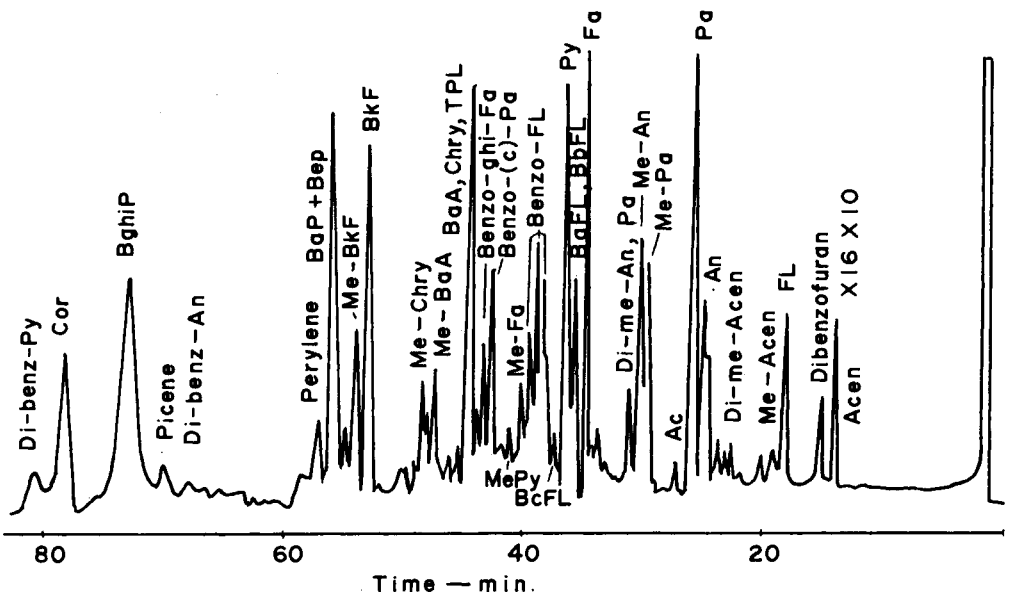

Fig. 3. Gas chromatogram of stack gas sample on Dexsil-300 WCOT column.

ed using a standard U.S. Environmental Protection Agency Method 5 sampling train (1971). About 1000 m^3 of the stack gas was drawn through the sampler. The glass fibre filter was removed and extracted 24 hours in a soxhlet using cyclohexane. The second sample is weathered roofing tar. The sample treatment procedure has been described previously (Lao, 1978).

The third sample was drawn from a "pot room" exhaust of a non-ferrous metal production unit using a fibre glass filter. The tared filter was re-weighed after sampling and extracted with cyclohexane. The PAH were isolated by back extraction using dimethyl sulfoxide (DMSO) similar to the process developed by Natusch and co-workers (1978).

Each of the PAH fractions were carefully concentrated with a rotary evaporator to provide adequate concentrations suitable for the various GC detection limits, and aliquot samples were injected onto each of the various columns for evaluation. Both 1 and 10 µl syringes were manufactured by Hamilton Co., U.S.A., and pretested with spectrograde solvents to verify their freedom from contamination. Sample volumes injected varied from 0.2 to 5 µl depending on column used and sample concentration.

Table 5 shows the typical PAH recoveries using the DMSO methods.

TABLE 5 Percentage Recovery of PAH Using DMSO

Compound	Natusch (1978)	Present Work
Fluoranthene	---	98
Pyrene	99.9	99
Chrysene	99.9	97
BaP	---	98

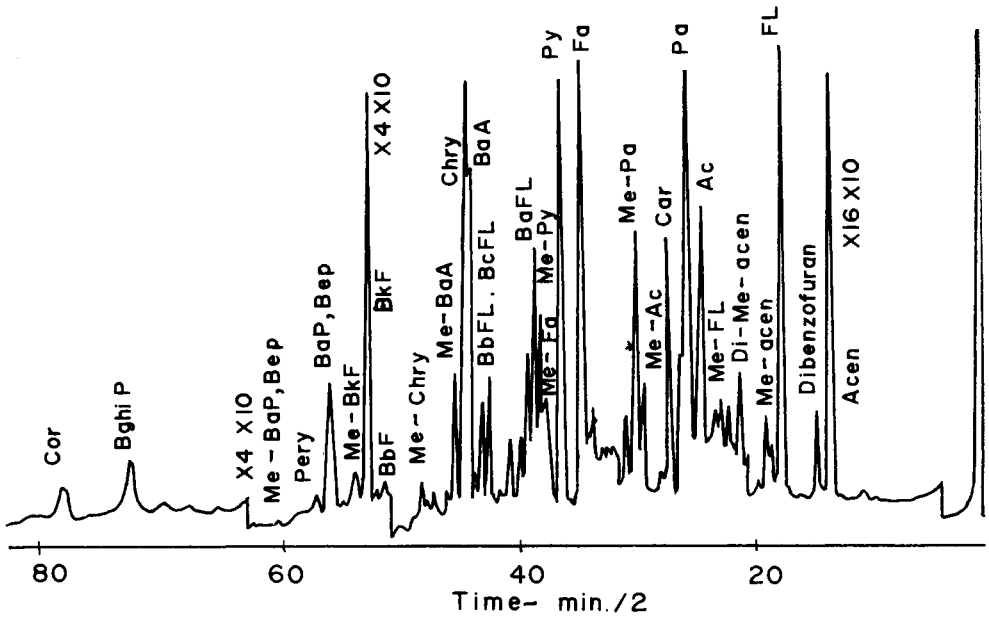

Fig. 4. Gas chromatogram of roofing tar extract on Dexsil-300 WCOT column.

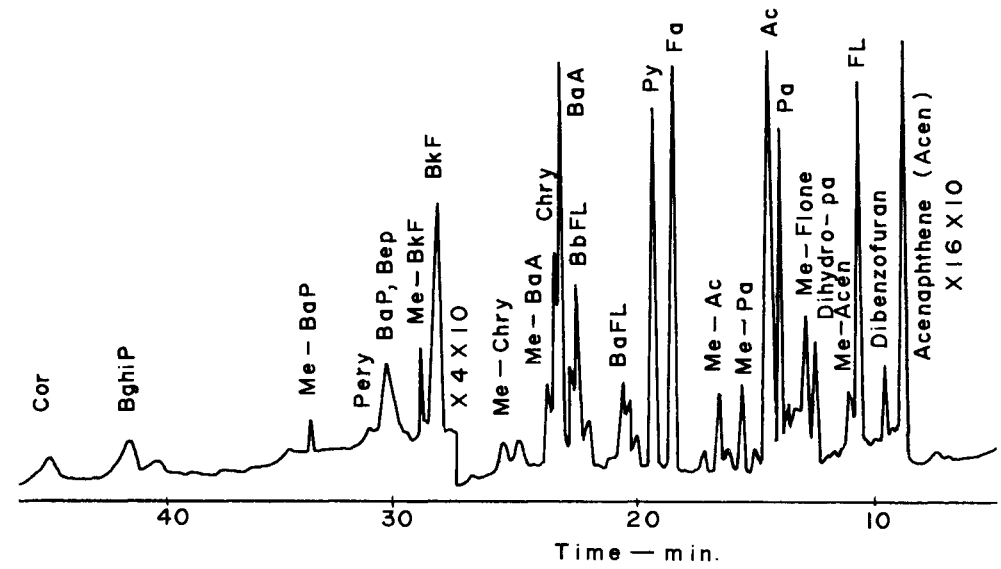

Fig. 5. Gas chromatogram of roofing tar extract on Dexsil-300 packed column.

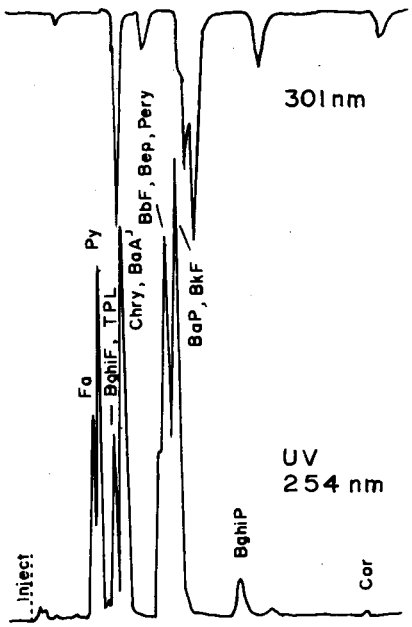

Fig. 6. Liquid chromatogram of 13 PAH on Microbondapak column; 70% CH_3CN/30% H_2O.

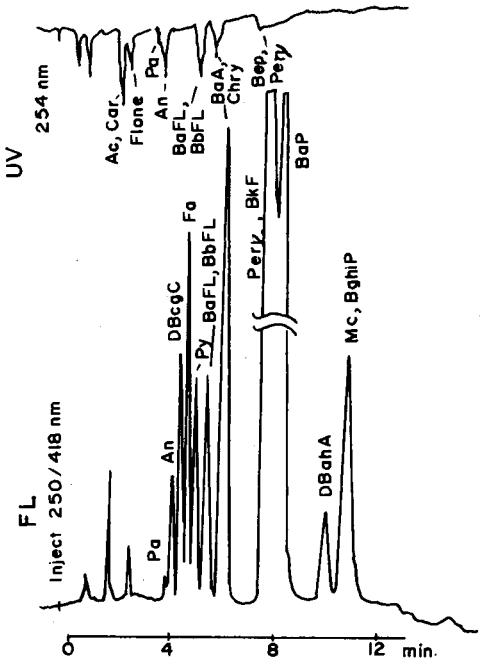

Fig. 7. Liquid chromatogram of 21 PAH on Microbondapak column; 70% CH_3CN/30% H_2O.

The extraction procedure was also tested for alkyl derivatives of PAH. The results indicated that the recovery percentage was only about 70-75%. The DMSO procedure may have to be modified before its application to this class of PAH compounds can be utilized.

RESULTS AND DISCUSSION

Figures 1 and 2 show the gas chromatograms of the 13 and 21 PAH standards using a Dexsil-300 capillary column. Figures 3 and 4 are the gas chromatographic traces for the stack gas sample and roofing tar extracts using a Dexsil-300 WCOT column. Figure 5 is the gas chromatogram of the roof tar sample using a Dexsil-300 packed column. From two prepared standard blends, the glass capillary column resolves chrysene from BaA and BeP from BaP. Triphenylene and chrysene however, remain unresolved. The chromatograms agree well with those of previous studies (Lao, 1973, 1978) on the Dexsil-300 and OV-7 packed columns. The WCOT column resolves BaA and chrysene but the separation of BaP from BeP is incomplete. By careful optimization of the GC parameters the WCOT column can partially resolve these two isomers (Lao, 1979).

The chromatogram from the split injection WCOT evaluation shows no significant improvement over the corresponding run of the same sample on the packed column. Nominal advantages of lower operational temperature,(Lao,1979) shorter retent-

ion time and moderate sample capacity are negated by price and availability compared with packed columns.

Figure 6 shows the separation of 13 PAH blend by HPLC and Figure 7 is the liquid chromatogram of the 21 PAH blend. The chromatogram in Figure 6 illustrates the results of optimized UV absorption wavelength selection at 254 and 301 nanometers.

The operational parameters for the HPLC system are similar to those previously reported (Lao, 1978) except that the solvent program has been linearized. The resolution is reduced and chrysene is not separated from BaA, and BbF, BeP and perylene elute as a single peak. In Figure 7, the fluorescence detector wavelengths are set at 250 nm excitation and 418 nm emission. The excitation wavelength is fixed. The relative increase in sensitivity of optical fluorescence detection of PAH over UV absorption technique is demonstrated. As in the previous study (Lao, 1978), selective BaA absorption at 290 nm provides a means for differential quantitation of BaA and chrysene. A similar situation exists for perylene and BeP; 290 nm gives absorption from BeP and perylene exhibits strong fluorescence at excitation wavelength of 365 nm.

Figure 8 is the liquid chromatogram for the stack emission sample previously used to evaluate Vydac column packings (Lao, 1978). A number of the components (peak Nos. 13, 14 and 15) remain unidentified since it could not be discerned if these peaks were single PAH components or unresolved mixtures. This ambiguity is particularly true when samples contain high percentages of alkyl derivatives whose electronic transitions at wavelengths are close to their parent compounds. Positive qualitative HPLC analyses for all the components of a complex environmental sample is only possible by the availabilitv of efficient HPLC/MS systems.

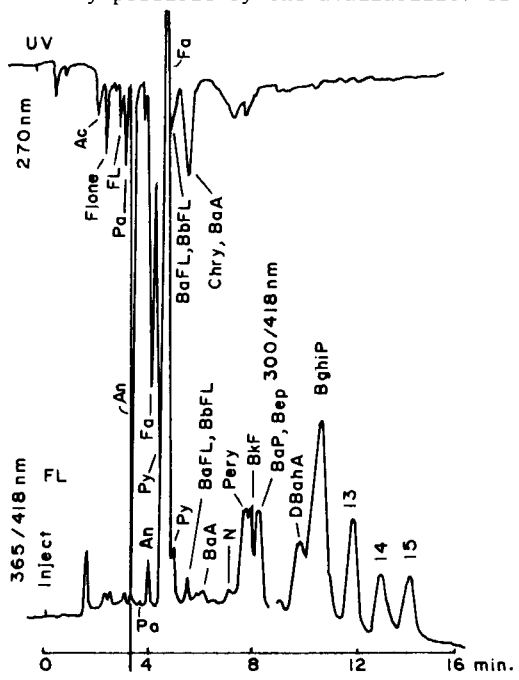

Fig. 8. Liquid chromatogram of stack emission sample on Microbondapak column; 70% CH_3CN/ 30% H_2O.

BKF and BaP do not separate at the 60/40 acetonitrile to water solvent ratio. If an initial solvent ratio of 75/25 is used, these two compounds begin to separate as shown in Figure 9. This isomeric resolution shows marked improvements for progressive decreases in water content of the solvent towards 10%.

Figure 10 is the computer reconstructed chromatogram from the Finnigan GC/MS system of pot-room exhaust. The sample has been analyzed (Lao, 1978) and some of the mass spectra of the individual chromatographic peaks are shown in Figures 11, 12 and 13. Figure 11 is the mass spectrum of GC peak No. 126 of Figure 10, fluoranthene, with mass/charge ratio (m/e) of 202 for the parent ion. Figure 12 is chrysene with m/e of 228, and Figure 13 is BaP of m/e 252.

Figure 14 is the result of the same exhaust sample using the Dexsil-300 glass capillary column, the analytical details have been described (Lao, 1979). Glass capillary columns can provide the highest resolution of all columns evaluated (Ettre, 1973; Jennings, 1978). Unfortunately, a restrictive device is often needed to avoid over-loading the column with solvent. The column capacity of about 5 x 10^{-3} microliter of sample requires a split ratio of 1:150 on injection and causes serious limits for detection of a number of the PAH present in an environmental sample. Recent developments in splitless injection techniques, column packings and pressure programming (Grob,1978; Onuska, 1978; Zlatkis, 1978) may eliminate many of the technical problems in glass capillary column operation.

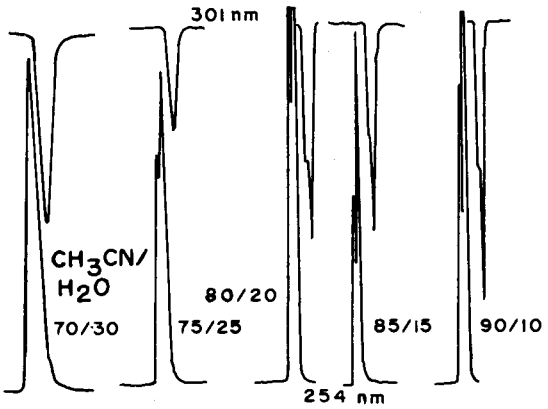

Fig. 9. Liquid chromatogram of BaP/BKF mixture on Microbondapak column.

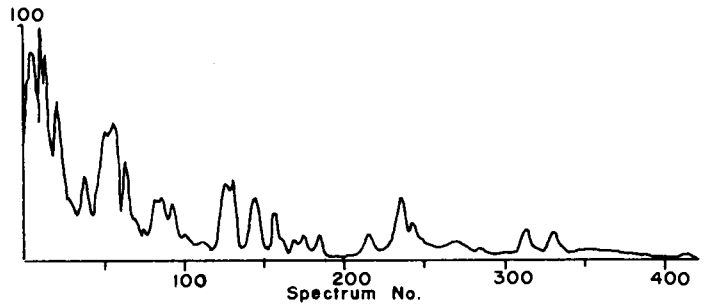

Fig. 10. Computer reconstructed chromatogram of pot-room exhaust.

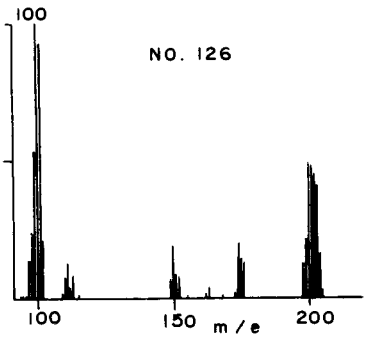

Fig. 11. Mass spectrum of GC peak No. 126 in Fig. 10.

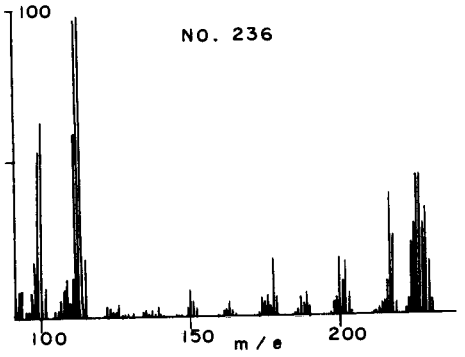

Fig. 12. Mass spectrum of GC peak No. 236 in Fig. 10.

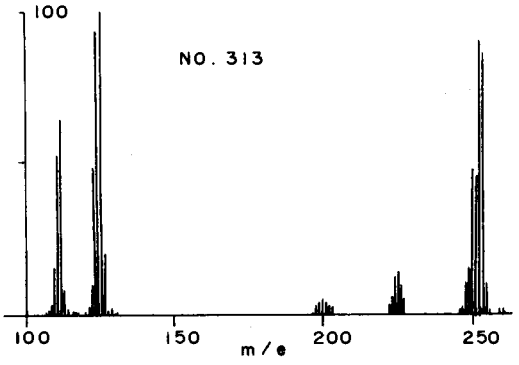

Fig. 13. Mass spectrum of GC peak No. 313 in Fig. 10.

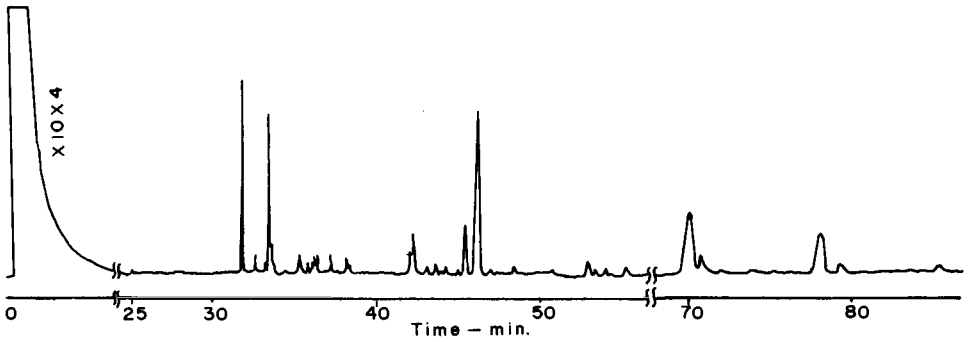

Fig. 14. Gas chromatogram of pot-room exhaust on Dexsil-300 glass capillary column; details see ref. Lao, 1979.

The PAH analysis time per sample is faster for HPLC than existing GC/MS methods. In addition, HPLC has higher selectivity, generally fewer interferences and shorter training time for the operator and lower capital cost. Although HPLC resolution is often low, it can be improved by recycling the sample through the analytical column; by optimizing the solvent polarities for individual isomeric PAH separations. Since aliphatic compounds show little UV absorption in the analytical wavelength region for PAH, complex environmental samples do not require the complicated clean-up procedures to remove interferences associated with GC techniques for PAH. The availability of highly sensitive optical fluorescence spectrometric detection systems and the relatively large sample load capacity of HPLC columns compared to GC columns of equal resolution have extended detection thresholds for PAH compounds. For example, the detection limit of BaP by GC is about 10 ng per injection, and 100 ng by Finnigan 1015D GC/MS system, whereas by HPLC the limit can be extended to about 10 pg.

In spite of the increasing use of HPLC for PAH analysis, GC/MS remains the primary method for the separation and measurement of PAH from environmental samples. One of the major disadvantages to use GC for evaluation of PAH has been the non-selective FID characteristic which measures all potentially interfering aliphatics and heterocyclic compounds together with PAH in samples. Continual refinement of column design, packings and liquid phases coupled with advances in pre-concentration and UV/IR detectors will re-define GC resolution and extend the sensitivity of the GC techniques(Novotny, 1976).

CONCLUSIONS

The development of class separation schemes based on solvent back-extraction techniques can provide a simple clean-up procedure for PAH compounds in environmental samples thus providing an efficient replacement for the widely used Rosen separation or other long procedures (Hoffman, 1978). GC/MS,HPLC and HPLC/MS represent current state-of-the-art analysis technology. However, problems of specificity and sensitivity for PAH measurement have not been completely solved. The sampling techniques for the PAH compounds in various media is neglected and becomes a major source of overall analytical error.

The analysis of trace PAH in the environment remains a challenging task and "there is no existing method which separates and resolves adequately the entire PAH fraction on a mg to μg scale" (Blumer, 1974). This is still true in 1978.

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Profiles of Polycyclic Aromatic Hydrocarbons in Suspended Particles

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ABSTRACT

An analytical procedure for the quantitative measurement of polycyclic aromatic hydrocarbons (PAH) based on the direct fluorometric scanning on thin-layer plates is presented. This method, which proved to be superior to gas chromatographie (GLC) with respect to the detection limit,was used in the analysis of particulate matter collected at various sampling stations in cities and rural districts with the aim of recognizing the influence of certain emission sources on the atmosphere by means of the PAH profiles,i.e. the relative proportions of the PAH. The resulting profiles of samples collected during four winters and one summer were very similar although the atmospheric concentrations of the PAH at the sampling stations were distinctly different.The rather uniform PAH profiles may be due to the fact that the emissions are mixed in the atmosphere in a much greater extent than suspected up till now.

KEYWORDS

Polycyclic aromatic hydrocarbons,quantitative measurement, suspended particles, thin-layer chromatography, chromatogram analyzer, PAH profiles.

INTRODUCTION

In recent times gas chromatography is considered to be the most convenient and sensitive method for the determination of polycyclic aromatic hydrocarbons (PAH). When examining the level of PAH in biological materials, the detection of trace levels by means of gas chromatography failed since the detector response was too poor. That was the reason for developing a procedure based on thin-layer chromatography,a procedure which has established its value against other methods.Quantitative measurement -once a tedious work- has been substantially improved now that scanners are available.

Analytical method.For the preparation of the sample we apply the usual Soxhlet extraction with cyclohexane and in addition we concentrate the PAH by sublimation under vacuum when the particulate matter is collected on glass fibre filters.The solvent is completely substituted by propanol-2. The first separation of the PAH is done on Sephadex LH 2o with propanol-2 as eluens. The elution time amounts to 11 hours. The fractions were collected in a fraction collector. The next step is the thin-layer chromatography of the fractions on acetylated

cellulose; the plates were developed using dichloromethane/ethanol/water 10 : 20 : 1 according to Schaad (1969).
Figure 1 demonstrates the separation of an extract derived from airborne particulated and underlines the importance of a preliminary column chromatography. In the fractions one to five only PAH are present.

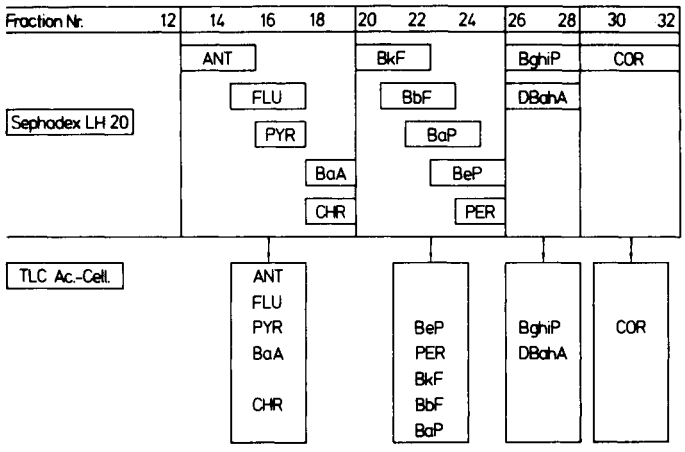

Fig. 1. Column chromatography and TLC separation of PAH in suspended particles

This facilitates the last step, the scanning of the spots with the chromatogram analyzer. The fluorescence intensity is recorded as a peak and an integrator totals the area under the recorder peak and converts the data to digital form. Fluorescence intensities differ from PAH to PAH. Chrysene and fluoranthene are the components with the weakest fluorescence while benzo(a)pyrene is very strong. As a result 4.4 ng of fluoranthene and 1.1 ng benzo(a)pyrene can be determined at a 1% level of significance.The standard deviation is 7% and 14% respectively (Tomingas,1977).When the sampling of the particulates is performed always with the same procedure regarding filter type, sampling time and air flow rate and a standardizised analytical method is used, we are able to estimate the detection limits of a method in dependence of the concentration of the PAH in the atmosphere. The result is shown in Fig. 2.
The estimation is based on the following conditions:
Sampling time 24 hours, total air volume 288 m, fraction volume 1 ml, 5o µl are spotted on the thin-layer plate. The content of any PAH on the spot is recorded on the abcissa, the atmospheric concentration on the ordinate. Conceding a detection limit of 5 ng for each PAH, a concentration of 0.35 ng/m^3 can be determined accurately. PAH concentrations in the range of 0.3 to 0.1 ng/m^3 are usually found in rural areas and demand prolonged sampling times.
PAH profiles. Since winter 1974 we have been collecting particulate matter on large glass fibre filters for animal experiments at various sampling stations which are located in cities and rural districts. Figure 3 shows the localization of the sampling stations in the western part of the Federal Republic of Germany. The rural district is represented by Krahm and Deuselbach, while Duisburg and Gelsenkirchen belong to the industrial centre. The level of air pollution is very different. We assumed a significant difference regarding the relations of the PAH to each other in the atmosphere of cities and rural areas.

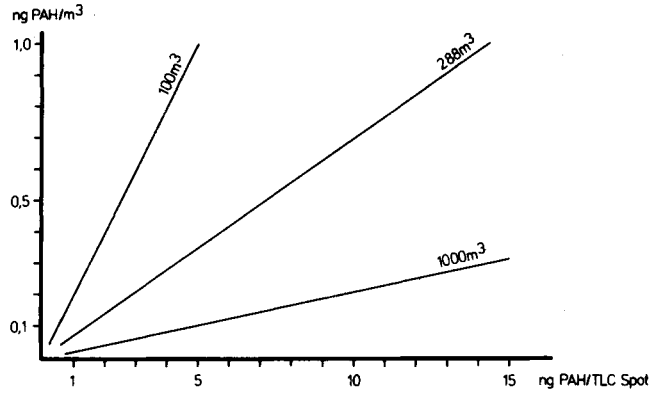

Fig. 2. Dependence of detection limits on sampling time and atmospheric PAH concentration for a definite sampling and TLC procedure

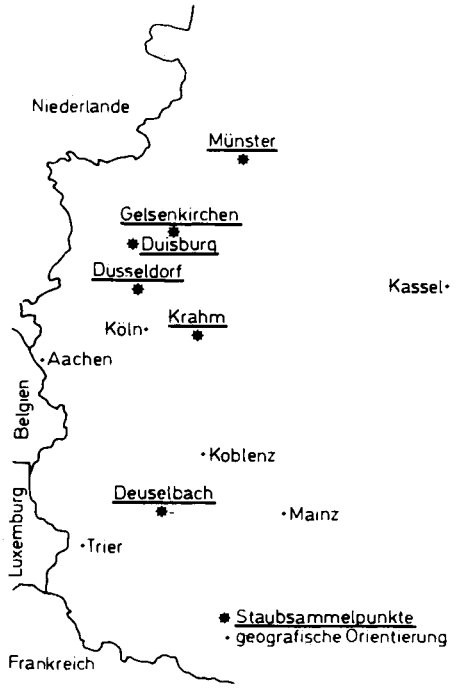

Fig. 3. Localization of the sampling stations

There was reason to believe that these different profiles of the PAH would give us an indication of the sources of emission. This opinion was strongly supported by the results of a former study where the PAH were determined in exhausts of domestic heatings (Brockhaus,1976). Figure 4 presents the PAH profiles with benzo(a)pyrene (BaP) as relative value. Eight PAH were analyzed: benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)-pyrene, perylene, dibenz(ah)anthracene, benzo(ghi)perylene, and coronene. Benzo(a)pyrene was set to 100,the relations of 7 PAH to benzo(a)pyrene were calculated and recorded in a log scale. It should be noted that all the graphs do not show a trail of agreement.

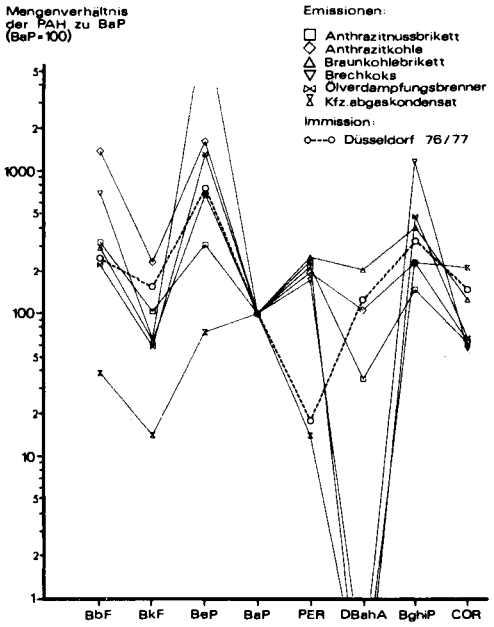

Fig. 4. PAH in exhausts of several kinds of coal, oil stoves,automobiles and suspended particles (BaP = 100)

Figure 5 demonstrates the PAH profiles in suspended particles of 5 sampling stations in the winter 1974/75 (Tomingas,1978). With one exception, and that is benzo(e)pyrene, the graphs are of the same shape. In order to decide whether this result was a mere accident we continued the investigation in the next winter.

Before that we compared the profiles of 2 sampling stations with those obtained in the summer 1975. Figure 6 proves with one exception-again benzo(e)-pyrene-that even in summer the PAH profile has not been altered. Figure 6 presents the situation in the winter 1975/76, it is exactly the same course as the graphs. Figure 7 demonstrates the profiles of the following winter.Obviously the values obtained from Münster do not fit into the general course. But the data from the winter 1977/78 in Fig. 8 reveal again profiles which are almost perfect in their uniformity.

On account of the log scale used for the graphic presentation, very low differences within the PAH profiles do not appear. To make even slight variations transparent, the correlation coefficients of 3 sampling stations were calcu-

ted. As Table 1 demonstrates, it now becomes evident that the correlation of the values derived from samples in winter 74/75 with all others is poor. But since summer 1975 up to the last winter the coefficients are within the range of 0.9 to 1. Thus, with one exception, the correlation coefficients are in accord with the graphs. To explain the values obtained in the winter 74/75 is difficult. In our opinion it may be due to the fact that in the Federal Republic during the following years coal burning was considerably reduced in favour of other fuels.

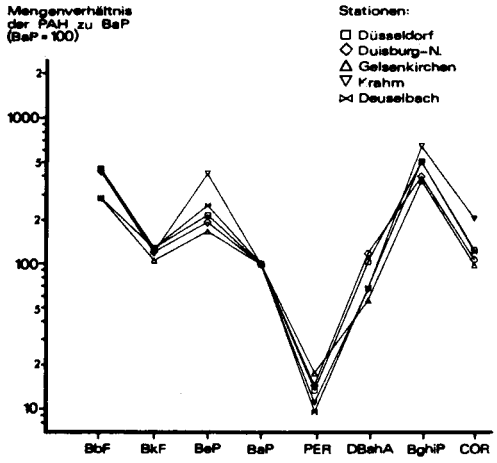

Fig. 5. PAH in suspended particles of 5 sampling stations; BaP = 100; sampling period winter 1974/75

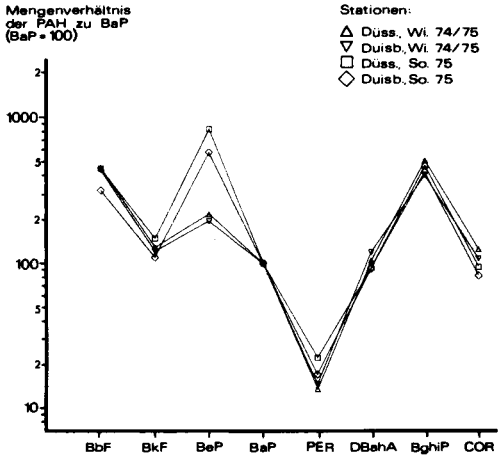

Fig. 6. PAH in suspended particles of 2 sampling stations BaP = 100; sampling period winter 1974/75, summer 1975

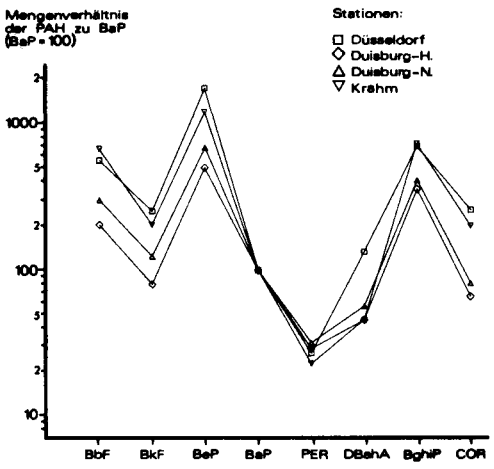

Fig. 7. PAH in suspended particles of 4 sampling stations BaP = 100; sampling period winter 1975/76

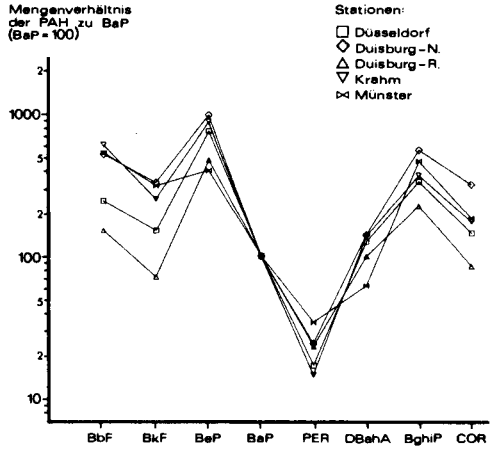

Fig. 8. PAH in suspended particles of 5 sampling stations BaP = 100; sampling period winter 1976/77

DISCUSSION

With the determination of 8 PAH in 4 sampling periods at 7 sampling stations there is, in view of these results, no opportunity of unmasking the sources of emission. However, the findings are surprising. It must be stressed, that the surroundings of the sampling stations strongly differ regarding the density of population, industry and traffic. Despite that, the conformity of the PAH profiles is striking. The situation in Duisburg, Gelsenkirchen and Düsseldorf may be explanable on account of the short distance between the stations and meteorological conditions.

But the distance between Deuselbach and Duisburg is 260 km and the correlation of the profiles with 3 cities is excellent. For this phenomenon we have at present no satisfactory explanation. We are of the opinion, that the mixture

of the emissions in the atmosphere leads to a rather uniform profile of the 8 PAH in the immissions as much as the difference in the stability of the PAH.

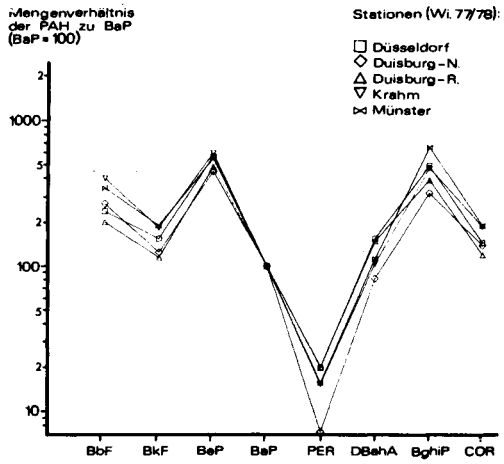

Fig. 9. PAH in suspended particles of 5 sampling stations BaP = 100; sampling period winter 1977/78

TABLE 1. Correlation Coefficients of PAH-Profiles in suspended Particles from several Sampling Periods for each Sampling Station

Sampling periods correlated	Sampling stations		
	Duisburg	Düsseldorf	Krahm
1974/75 - Su 75	0,67	0,62	—
- 75/76	0,56	0,42	0,82
- 76/77	0,57	0,41	0,71
- 77/78	0,67	0,67	0,90
1975/76 - Su 75	0,99	0,97	—
- 76/77	0,96	1,00	0,96
- 77/78	0,97	0,90	0,98
1976/77 - Su 75	0,94	0,96	—
77/78	0,98	0,92	0,93

Regarding the stability of the PAH the question arises whether the resemblance of the profiles depends on the sampling procedure only. The profiles presented here were derived from a particulate matter sampled in a period up to 120 days. Undoubtedly losses of PAH occur during the sampling period. Therefore the influence of the sampling time on the relative proportions of the PAH in suspended particles was examined thoroughly. We varied the sampling time, the sampling stations, the sampling device, and even the techniques of enrichment. As a result, the difference to the profiles already shown is but trifling. An example is given in Fig. 10. The particulate matter was sampled in Düsseldorf

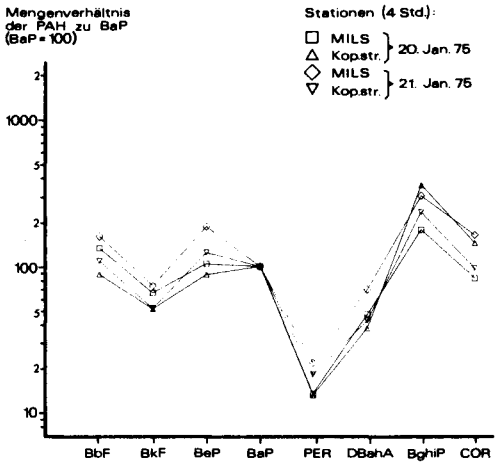

Fig. 1o. PAH in suspended particles of 2 sampling stations; sampling time 4 hours

at 2 sampling stations within 4 hours only on glass fibre filters 9 cm in diameter. The PAH were concentrated by means of sublimation under vacuum. The graphs show the already well known course.
Further investigations will prove whether the profiles will change in the following years. The results within a period of 4 years seem to justify the assumption that the relations of the PAH are fairly constant. Finally, a distinct alteration of the PAH profiles will indicate a large change of the emission site.

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Method Development and Monitoring of Polynuclear Aromatic Hydrocarbons in Selected U.S. Waters

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ABSTRACT

Six representatives of the polynuclear family were recovered from trace levels in water by sorption on polyurethane foam plugs. Optimum conditions for recovery included heating the influent water to 62° ± 2°C and maintaining a flow rate of 250 ± 10 ml/min. The collected PAH were subjected to a clean-up procedure and analyzed by two dimensional thin layer chromatography coupled with fluorometric detection. Gas liquid chromatography-flame ionization failed to detect PAH at low concentrations and in some instances did not provide adequate resolution of different PAH. PAH were detected in trace quantities in all the water supplies studied. Whereas the sum of the six representative PAH in drinking water was small (0.9 - 15 ppt), the values found in raw water were as high as 600 ppt. It is unclear whether PAH are removed during treatment or whether they are transformed to another product and escape detection. The levels of PAH in water increased after contact with the distribution network. An increase in the mutagenic activity of water in the Ames assay was also noted at many sites following passage through the distribution pipes. Some of the mutagenic activity was suspected to be due to PAH.

KEY WORDS: Polynuclear Aromatic Hydrocarbons, polyurethane foam, water treatment, distribution network, raw waters, drinking waters.

INTRODUCTION

Industrialization of our civilization has resulted in an increased contamination of our environment with a number of cancer producing chemicals. Polynuclear aromatic hydrocarbons are of particular concern because of their demonstrated carcinogenic activity (IARC, 1973; Suess, 1970), wide distribution, and persistence in the environment (Radding and co-workers, 1976). The potential hazards from the occurrence of PAH in man's water supplies have been noted by the World Health Organization's Committee on the Prevention of Cancer which recommends evaluation of the treated surface waters for PAH (WHO, 1971).

The major environmental sources of PAH are technological processes involving combustion or pyrolysis of material, such as heat and power generation, refuse burning, miscellaneous industrial processes and emissions from vehicular transportation media. PAH in small quantities also appear in the environment from natural sources such as forest fires, volcanic activities and endogenous synthesis by some living organisms (Suess, 1976). These carcinogenic substances may enter natural waters, and thereby public water supplies, in a variety of ways including the release of industrial effluents, direct fallout from atmospheric particulate matter, road run-offs, discharge from urban and domestic sewage, and run-off or leaching from soils.

The studies on the incidence of PAH in the water environment have been carried out predominantly in Europe (Borneff, 1977; Acheson and co-workers, 1977) and are pertinent to European waters only. Data regarding their levels in U.S. waters is virtually non-existant. The present study was undertaken to determine the concentration of PAH in selected raw water sources, their degree of removal during treatment, and contribution of PAH, if any, from water distribution pipes which are frequently coated with coal tar or petroleum base sealant.

EXPERIMENTAL

The six PAH included in the study are: benzo(a)pyrene (BaP), fluoranthene (FL), benzo(k)fluoranthene (BkF), benzo(j)fluoranthene (BjF), indeno(1,2,3-cd)-pyrene (IP), benzo(ghi)perylene (BPR). Benzo(b)fluoranthene, which is recommended by WHO for use in analytical studies, has been replaced with benzo(j)-fluoranthene in our analysis due to the non-availability of BbF.

PAH from water were recovered by filtration through flexible polyurethane foam plugs (45 x 45 mm, foam density 25 kg/m^3) as reported earlier (Saxena, Kozuchowski and Basu, 1977; Basu and Saxena, 1978). The method involved passing water through foam plugs at a temperature of 62° $\pm$ 2°C and flow rate of 250 $\pm$ 10 ml/min. Four foam plugs placed two each in two different columns and connected in series were sufficient to retain PAH from 20ℓ of treated- or 10ℓ of raw water. A sampling apparatus allowing maintenance of optimum conditions in the field was assembled within a suitcase (Fig. 1). The unit consisted of a Haake water heater, thermostat, appropriate columns for holding plugs, and a variable speed pump.

Following concentration of PAH at the sampling site, foam plugs held in the glass columns were brought to the laboratory for analysis. The entire sampling and analysis procedure is shown schematically in Fig. 2. The sampling volume in the studies described here was 60ℓ for treated water and 30ℓ for raw water.

The detection limit for individual PAH with these sample volumes was calculated to be in the range of 3-5 ppt for gas liquid chromatography - FID detection, and 0.1 - 2 ppt for thin-layer chromatography-fluorometric detection. Measurements of the levels of PAH by two independent methods allowed cross checking of the data. Although both methods were employed in these studies, data is given here only for TLC-fluorescence, since GLC-FID in many instances provided inadequate resolution of the PAH and/or failed to detect them.

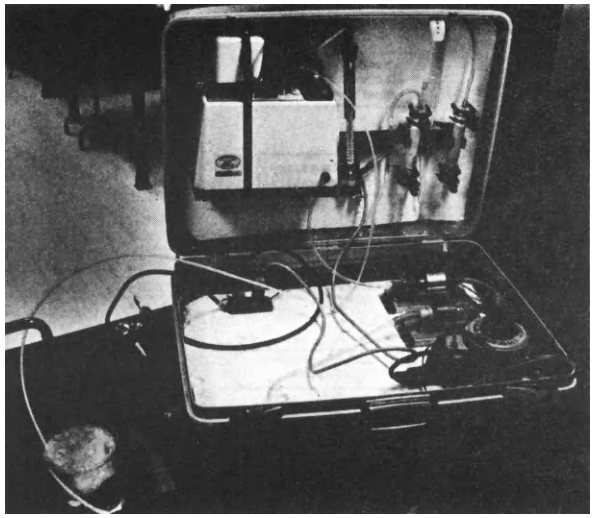

Fig. 1. Apparatus used for recovering PAH from water

RESULTS AND DISCUSSION

Foam retention efficiencies for the six PAH from spiked laboratory tap water are shown in Table 1. The data showed that under the conditions employed, polyurethane foam plugs effectively concentrate PAH from water. The high PAH retention efficiencies were also maintained with heavily polluted surface water (Table 2). The actual concentration of each PAH used in the foam retention studies was determined by extraction of an aliquot of the spiked

TABLE 1. Foam Retention Efficiencies for PAH from Treated Water. Water Source: Laboratory Tap Water; Water Volume: 60 L; Concn of Fluoranthene: 500 ppt; All Others: 100 ppt; Detection Method: TLC-Fluorometric

Compound	Concn in aqueous phase (ng/L)	Amt retained by foam from 1 L of water (ng)	% Retention
FL	278.6	260.4	93.5
BjF	48.3	47.4	98.1
BkF	51.7	50.6	97.9
BaP	36.4	33.6	92.3
IP	25.5	23.9	93.7
BPR	22.6	19.8	87.6

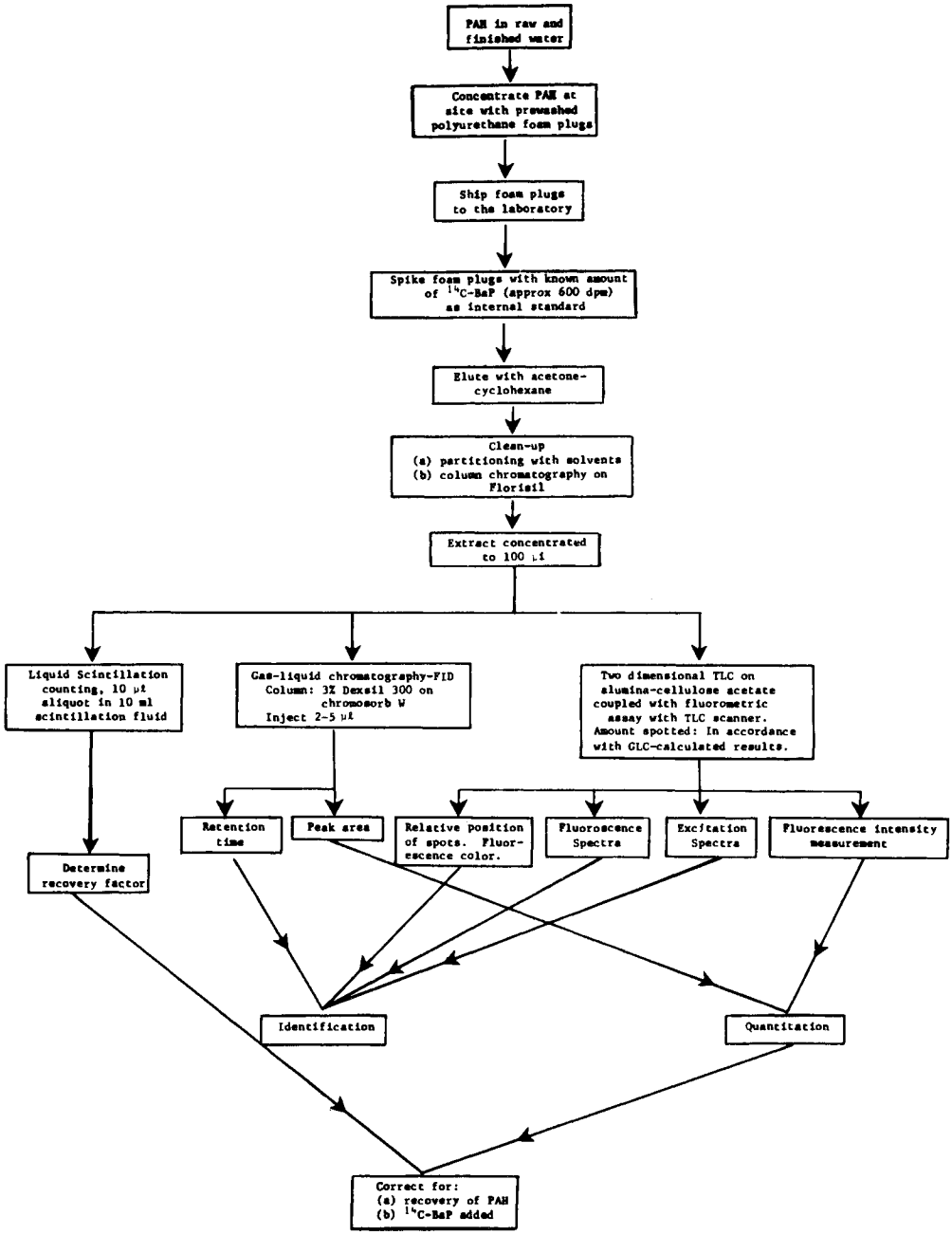

Fig. 2. Flow Chart of the Method of PAH Analysis in Water

water with cyclohexane. The greater than 100% retention values seen for FL, BjF, and BPR may possibly be due to lower recovery of these PAH by cyclohexane, compared to foam. This is supported by the findings of Acheson and co-workers (1976), who have shown that extraction efficiencies of organic solvents vary for different PAH.

TABLE 2. Foam Retention Efficiencies for PAH from Raw Water. Water Source: Onondaga Lake; Water Volume: 30 L; Concn of Fluoranthene: 500 ppt; All Others: 100 ppt; Detection Method: TLC-Fluorometric

Compound	Concn in aqueous phase (ng/L)	Amt retained by foam from 1 L of water (ng)	% Retention
FL	289.1	343.7	118.9
BjF	77.6	94.0	121.1
BkF	66.1	55.6	84.1
BaP	74.5	59.7	80.1
IP	85.2	61.2	71.8
BPR	23.9	28.3	118.4

The method devised was applied to monitoring PAH in selected U.S. drinking waters and their raw water sources. Since one of the objectives of the study was to elucidate the effect of water treatment processes on the levels of PAH, drinking waters were sampled at the treatment site. This precluded changes in PAH concentrations caused by the water distribution process. PAH were detected in the ppt range in both raw and finished water at all the locations sampled (Table 3). While the concentrations of the six PAH used as standards was small in drinking waters (0.9 to 15 ppt), concentrations as high as 600 ppt were found in raw waters. In many water supplies, all six WHO chosen representatives of PAH were detected. Polynuclear compounds such as BaP, BPR and B(k)F were among the most frequently occurring PAH. Surprisingly, fluoranthene, which is relatively more water soluble than the other five selected members of the group, was not widely detected. Comparison of the levels of PAH in finished and raw water revealed considerable reduction in concentration during water treatment. It is not known if the decrease was due to actual removal, or to transformation of the PAH to other products which may not be retained efficiently on the foam plug. If the observed reduction in PAH concentration is due to transformation, then the possibility exists that such conversion of PAH may result in formation of compounds more carcinogenic than the parent compound.

Finished water from the treatment site is transported to consumers through pipelines which are frequently coated with coal tar or petroleum base sealant, which could be potential sources of PAH in water. On the other hand, PAH could be adsorbed from water onto the surface of some type of pipes. To determine the effect of water pipes on the concentration of PAH, drinking waters at the treatment site and at two points in the distribution system were analyzed for the presence of PAH. One of the sampling points selected was the most distant practical point representing maximum exposure to the

pipe, and the second point was at approximately intermediate distance in the system.

TABLE 3 Levels of PAH in Raw Waters and Their Removal/Transformation during Water Treatment. Treated Waters were Sampled at the Treatment Site.

Water Supply System	Treated/Raw Water	PAH Detected						
		FL	BjF	BkF	BaP	IP	BPR	Total of the Six PAH
		Parts per trillion						
Buffalo, N.Y.	Raw water	-	-	0.6	0.3	-	3.8	4.7
	Treated water	-	-	-	0.2	-	0.7	0.9
Huntington, Va.	Raw water	23.5	5.0	3.6	5.6	9.5	10.7	57.9
	Treated water	2.4	0.3	0.2	0.5	1.2	2.5	7.1
Philadelphia, Pa.	Raw water	114.3	42.6	33.0	41.1	72.4	48.4	351.8
	Treated water	8.9	0.3	1.7	4.0	-	-	14.9
Pittsburg, Pa.	Raw water	408.3	35.7	19.1	42.1	60.4	34.4	600.0
	Treated water	-	0.3	0.2	0.4	1.2	0.7	2.8
Name withheld*	Treated water	-	1.2	0.7	0.5	2.2	1.8	6.4

* Northeast location, referred in the text as NE.

Many of the distribution systems studied caused increases in the levels of some PAH accompanied with an increase in the sum of the six PAH over the level determined in water at the treatment site (Table 4). Several points become clear from the data. At Appleton, and at the location abbreviated as CH, BPR and IP were the major PAH introduced from the distribution lines. The finished water at Elkhart and Fairborn showed trace quantities of some PAH; however, after exposure to the distribution pipes, all the 6 PAH were detectable in these waters. At a few locations, the levels of PAH decreased after exposure to the distribution system. This suggests the possibility of removal of some PAH by adsorption on the surface of certain kind of pipes. Efforts to link the PAH levels to the nature of the coating within the distribution pipes were unsuccessful because definitive information regarding the coating used at these locations could not be obtained.

The potential of distribution lines as contributors of PAH and perhaps other mutagens/carcinogens was further investigated utilizing the Ames Salmonella assay (Ames, 1975; Saxena and Schwartz, 1979). The assay involves the use of specially constructed histidine requiring mutants of S. typhimurium and is capable of detecting a wide range of mutagens/carcinogens at low concentrations. The samples taken at the treatment plant and at two locations within the distribution system at Appleton, Wheeling and at location CH were tested for mutagenic/carcinogenic potential. The distribution network at CH introduced two different types of mutagens into water. One tested positive for mutagenic activity in the base pair substitution strain TA-100 without metabolic activation and could not be adsorbed on polyurethane foam. The second type tested positive in the frameshift mutant TA-98, required metabolic activation and could be recovered by adsorption on foam. At Appleton,

TABLE 4 Effect of the Water Distribution Process on the Levels of PAH

Water Supply System	Sampling Location	PAH Detected						Total of the Six PAH
		FP	BjF	BkF	BaP	IP	BPR	
Appleton, Wis.	a. Treatment site	-	0.4	0.2	0.4	1.4	3.7	6.1
	b. 1.6 miles from treatment site	-	0.4	0.3	0.3	1.4	5.4	7.8
	c. 3.5 miles from treatment site	-	1.4	0.9	1.2	3.3	11.2	18.0
Fairborn	a. Treatment site	-	-	-	0.1	0.7	2.1	2.9
	b. 2.5 miles from treatment site	-	-	-	0.4	-	-	0.4
	c. 4 miles from treatment site	6.5	0.3	0.3	0.3	0.6	0.6	8.6
Elkhart, Ind.	a. Treatment site	-	-	-	-	0.3	-	0.3
	b. 1.5 miles from treatment site	8.1	0.4	0.3	0.3	0.6	1.4	11.1
Wheeling, W.Va.	a. Treatment site	94.5	1.4	-	2.1	7.8	32.7	138.5
	b. 4 miles from treatment site	81.7	0.8	-	0.8	3.7	14.1	101.1
	c. 12 miles from treatment site	-	1.4	-	1.9	4.9	21.6	29.8
Name witheld *	a. Treatment site	-	0.3	0.3	-	0.1	1.3	2.8
	b. 5.3 miles from treatment site	-	0.6	0.5	0.5	1.1	3.5	6.2

* Midwest location, referred to in the text as CH.

only the mutagens of the former type were detected whereas Wheeling water tested out to be non-mutagenic. The mutagenic and sorptive properties of the second type of mutagen(s) suggest that these compounds could be PAH. However, no direct correlation was seen between the mutagenic activity of the waters studied and the total concentration of the 6 representative PAH. For example, the water samples at location CH showed strong mutagenic response but only trace quantities of the six PAH. On the other hand, Wheeling water which contained the highest concentration of the six PAH was non-mutagenic. This suggests that the distribution network contributes PAH other than the six measured, or some mutagens which are not PAH.

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A Novel Luminescence Instrument for Rapid Identification and Monitoring of Aromatics in Environmental Chemistry

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ABSTRACT

Luminescence methods find increasing use in environmental chemistry because of high sensitivity and specificity for aromatics. Usually mixture analysis involves repetitive scanning and interpretation of the numerous data by a skilled scientist, limiting the utility of the technique. The instrument described will allow rapid acquisition and interpretation of the "total luminescence" of a sample, allowing full exploitation of the potential of luminescence analysis.

We define total luminescence as the intensity function, $I(\lambda_{ex},\lambda_{em})$, where λ_{ex} and λ_{em} are all possible excitation and monitoring wavelengths. The optical head, employing crossed polychromators, produces the intensity function, I, as a real image in space, thus providing full luminescence information in real-time. A video detector is coupled directly to a fast minicomputer for immediate correction, analysis and display of data. Correlations with model spectra can be performed quickly, allowing rapid identification. This speed allows direct coupling to a chromatograph.

Potential applications of this new instrument in various environmental areas are discussed with an example of mixture analysis of nitrogen heterocyclics.

Keywords: aromatic, detector, environment, fluorescence, identification, instrumentation, luminescence, monitoring.

INTRODUCTION

Background

Luminescence theory and methodology have a long history of development which has culminated in numerous practical applications in the fields of clinical chemistry, pharmacology, forensic chemistry, pesticide analysis, and environmental monitoring. Newer methods utilizing room temperature phosphorimetry, lasers and other novel techniques are reported regularly. Application to identification and characterization of petroleum oils has become common, resulting recently, in the promulgation of an ASTM method.

Single Compound Analysis

For a single molecular species the observed intensity, I, is a function of both the excitation wavelength, λ_{ex}, and the monitoring wavelength, λ_{em}. For

the vast majority of aromatic molecules emission occurs from the lowest excited triplet or singlet states. As a result the intensity function is separable, i.e. it can be written as a product of functions of one variable:

$$I(\lambda_{ex},\lambda_{em})=\alpha X(\lambda_{ex})\cdot M(\lambda_{em}) \quad (1)$$

where X and M are proportional to the excitation and emission spectra respectively and α is an instrumental constant. Therefore all luminescence information is contained in a single excitation spectrum together with a single emission spectrum.

Simple Mixture Analysis

For a dilute (non-interacting) mixture of luminescent molecules the observed emission becomes a sum:

$$I(\lambda_{ex},\lambda_{em})=\alpha\Sigma_i X_i(\lambda_{ex})\cdot M_i(\lambda_{em}) \quad (2)$$

where the index i identifies the molecular species. Since the observed intensity is no longer a simple product, the emission spectrum will depend on the choice of excitation wavelength (and the converse). To acquire full luminescence information it is necessary to produce a large number of emission races, each for a different excitation wavelength, or a large number of excitation traces, each for a different monitoring wavelength. Such voluminous data are not only tedious to produce with standard scanning spectrofluorometers, but they are difficult to analyze visually. These difficulties have hindered the exploitation of the full potential of luminescence measurements for mixture analysis.

Total Luminescence Spectroscopy

We have coined the term Total Luminescence Spectroscopy (TLS) to describe the acquisition, display, and interpretation of the full luminescence information available from a given sample. In Fig. 1 we have plotted the intensity, I, for a hypothetical sample as contours of equal intensity, with λ_{ex} and λ_{em} as ordinate and abscissa. Such a diagram contains the full luminescence information available from the given sample under experimental conditions. Referring to Fig. 1, the vacant triangular region at the upper left corresponds to $\lambda_{ex} > \lambda_{em}$, where no emission can occur. Rayleigh and Tyndall scattering are centered on the 45^0 line through the origin where $\lambda_{ex}=\lambda_{em}$. A standard excitation scan at a fixed monitoring wavelength corresponds to a vertical cut through the figure. Analogously, an emission scan at a selected excitation wavelength corresponds to a horizontal cut through the figure.

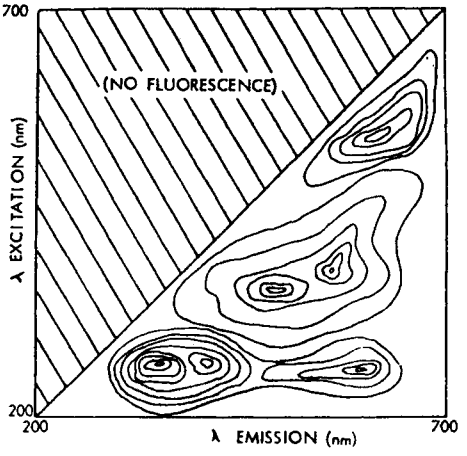

Fig. 1. Hypothetical total luminescence intensity contour diagram.

The total luminescence approach has been used at Baird for many years, particularly for petroleum studies, (Hornig and Brownrigg, 1975a, 1975b; Hornig, Eldering & Coleman, 1976; Hornig 1976; Brownrigg, Hornig & Coleman 1977; Giering & Hornig, 1977a, 1977b, 1978a, 1978b; Hornig & Giering, 1978).

Data to produce a total luminescence spectrum may be acquired by serially

scanning excitation or emission over the λ_{ex}-λ_{em} plane. This tedious process maybe speeded up by automation of scanning instruments; however, the ultimate solution is the design of a new type of spectrofluorometer which produces the intensity image in its exit plane and scans that image electronically. Such an instrument was first constructed by Warner & co-workers (1975).

INSTRUMENT DESIGN

General Description

The instrument is designed to allow all luminescence measurements normally carried out with typical scanning spectrofluorometers. The instrument is modular, allowing quick update as new components emerge. The primary requirement placed on the instrument is that it be able to acquire and process the total luminescence data in a time short enough to allow use as a detector for a chromatograph.

The instrument has two logical parts: the optics section includes the light source and power supply, crossed polychromator spectrometer, sample holders and fixtures, and array detector; the signal processing section includes a minicomputer system with large storage, a graphics display unit, and a hard copy output. Besides the hardware there is a basic software package which allows necessary data manipulation. A block diagram of the overall system is given in Fig. 2.

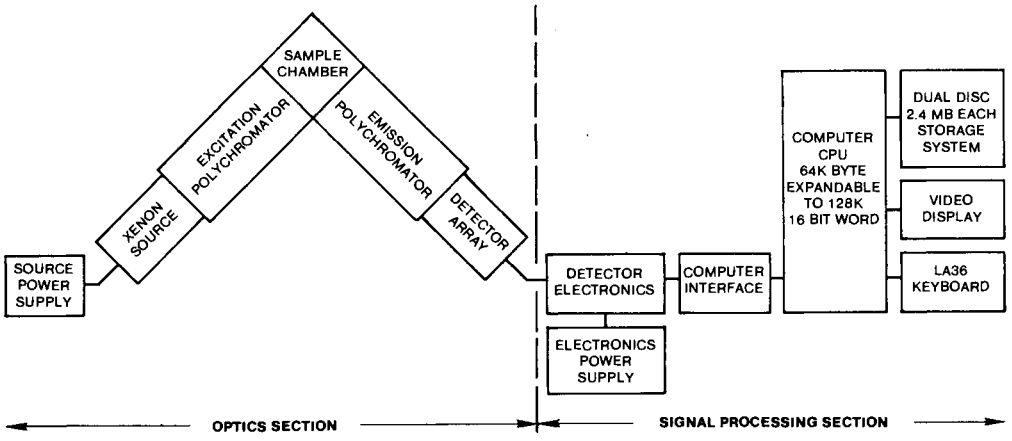

Fig. 2. Block diagram of total luminescence spectrometer.

Optics

Light sources. The primary light source should cover the near ultraviolet and visible portions of the spectrum with high brightness. In order to keep aberrations of the first polychromator to a minimum the source should be small in size, to permit use of a short entrance slit. We have selected a 150 watt dc xenon arc built by Varian/Eimac which produces an image with an F/3.5 cone.

Crossed polychromator spectrometer. The heart of the system consists of two concave holographic gratings, designed by Instruments SA, Inc. to produce flatfield images over wide spectral ranges. Because they are concave, several focussing mirrors can be eliminated from each polychromator, leading to greater simplicity and reliability. The excitation grating was designed to operate at F/3.5, which couples well with the xenon source discussed above. The spectral domain is 200-700 nm with a dispersion of approximately 16 nm/mm. The emission grating was

designed to operate at F/5. The spectral domain is 250-750 nm with a dispersion of approximately 13 nm/mm. The final total luminescence image dimensions are 32 mm for excitation and 38 nm for emission.

Detectors. Possible detectors include vidicons, charge-coupled devices (CCD's) and charge-injection devices (CID's). Based on the experience of the University of Washington group using a silicon intensified target (SIT) vidicon (Johnson, Callis & Christian, 1977), and on discussions with astronomers at Kitt Peak National Laboratory (Aikens, Lynds & Nelson, 1976) where both vidicons and diode arrays have been used, we have decided to use the best available diode array, believing this to be the area where future developments are occurring most quickly.

A problem with all detectors is resolution. Thus if a resolution of one nanometer is desired over a 500 nanometer domain, a minimum of 1500 pixels will be required in that dimension. Unfortunately arrays of this size are not available at this time. Therefore some combination of reduced domain and reduced resolution is necessary. Except at low temperatures and for certain materials, a resolution of one nanometer is not required. Further, the resolution to be obtained from the gratings at the corners of the final image is not expected to be one nanometer.

The largest commercially available arrays are a 320 x 256 pixel CCD from RCA and a new 244 x 248 pixel CID from G.E. We prefer a CID to a CCD for several reasons, one being the higher surface area responding to light (due to simpler geometric configuration), the other being the potential for selecting and interrogating individual pixels.

A second problem comes with coupling the large image (32 mm x 38 mm) with the rather small array (of the order of 10 mm). We propose to accomplish this coupling through use of fiber optics and a microchannel plate. A standard 40 mm microchannel plate from ITT will receive most of the optical information. The microchannel plate cathode material can be selected to preserve ultraviolet response. The optical output of the microchannel plate is then coupled to the array by an optical fiber bundle. With the GE CID array, a pixel will represent about 2 nm, producing a resolution of approximately 6 nm.

Mode of Operation. The excitation polychromator disperses light along the sample. The fluorescing sample is imaged on the entrance slit of the emission polychromator, leaving excitation wavelength encoded along the slit. The emission polychromator disperses fluorescence radiation at right angles to the excitation dispersion, resulting in an intensity image in the exit plane where the detector array is located. (See Fig. 1.)

Signal Processing

The selection of the optimum interface between the detector array and the computer, and the selection of the computer itself, are based on current and projected future requirements of the final instrument. Thus, the system must work with the 244 x 248 pixel CID detector array; however, it should also be expandable to handle the 800 x 800 CCD array which may be available in a few years. A single complete frame must be read out in a time which is but a fraction of the duration of a typical chromatographic peak. We have used one second as a goal. The system must allow repeated scans to allow signal averaging to increase S/N where necessary. Finally, the processing of the signal (including spectral correction and presentation as a contour or isometric projection) and the video display of the results must occur in approximately one minute to allow interactive decisions by the operator. The storage must be large enough, and expandable, to allow storage of large numbers of samples and standards.

Hardware. The Preston a-d converter selected requires about 1.5 microsecond per conversion. Allowing an additional 0.3 microseconds for video processing, the resulting data rate for the 60, 512 pixels comprising the 244 x 248 pixel CID is approximately 0.1 seconds per frame. This is well under the one second goal required for a chromatographic detector. If we look ahead to the 800 x 800 array, the frame time is increased by approximately an order of magnitude to approximately one second. This is still reasonable, although it may be desirable to cool the detector to reduce the dark current.

The choice of computer system depends strongly on the nature of the problem. In general the computer must accept the data rate above while processing data already in the computer. This is necessary to permit continual data-taking to improve S/N, while producing displays (e.g. contours) for immediate observation. Data processing for display includes correction for wavelength accuracy, intensity correction of the optical system as a function of wavelength, and calculation of contours. Initial consideration was given to a state of the art, 16-bit minicomputer such as the PDP-11. However both processing speed and addressing limitations forced investigation of other processors and architectures.

In the domain of 32-bit machines consideration was given to both the Digital Equipment Corp. VAX-11/780 and the System Engineering Laboratories 32/57. The latter was selected because of the ability to directly address up to 16MB of main storage and a bus cycle time of 150ns or 26.6Mb per second. The 900ns cycle time of main storage is augmented by instruction look-ahead and full memory overlap. Due to the extreme speed of the computer and the availability of the ISA subroutine library it will be possible to write nearly all the software in FORTRAN. This is a significant advantage since it makes future modification and enhancement of the system far simpler than if the software were written in assembler for a slower machine. These features combine to assure success at the proposed data rates and sufficient margin to permit growth as the state of the art advances.

Software. Key in our general software design is the Executive System and Command Languages, designed to permit simple, rapid addition of commands and processes as defined by the needs of the application. This scheme is particularly suited because extensibility of both commands and programs are inherent in the design.

The basic software program will allow the instrument to scan an image in under one second, transferring data to storage for further manipulation. Wavelength calibration and intensity correction will be automatic. The instrument will be able to perform basic mathematics (add, subtract, multiply, divide, take a log) on data files. The results can be plotted in a variety of ways for display on a graphics terminal, or on hard copy. The basic display will be contours, however, isometric projection will also be available. The instrument can be directed to plot any "cut" through the λ_{ex}-λ_{em} plane, including excitation, emission and synchronous scan. Automatic labeling of printed output will be possible.

While the finished instrument will be versatile, the user can look forward to a wide variety of new possibilities, many of which will develop as experience increases. Computer analysis of mixture spectra is one very important region. The basis for such analysis was laid by Weber (1961): most recently the University of Washington group (Warner and co-workers, 1977, Ho, Christian & Davidson, 1978) has extended the method specifically for computer use with a Total Luminescence Spectrometer. Undoubtedly it will be useful to accumulate a library of model compounds. Correlation programs can be written for automatic search and identification of an unknown. The annoying superposition of first and second

order spectra at longer wavelengths can be eliminated by automatic subtraction of second order spectra. Once the polarization characteristics of the instrument are determined, corrections can be added to memory and automatic polarization spectra can be developed. Basic studies on quenching and energy transfer can be carried out automatically with proper programming.

Coupling of the instrument to a chromatographic separation step will undoubtedly be a major use. Many of the above-mentioned studies can be carried out during chromatographic runs, due to the extreme speed of the computer.

APPLICATIONS

Basically the instrument is designed to acquire, digest and display very rapidly the total luminescence of a sample. The greatest number of luminescent materials in the environment are aromatics; hence this will be the largest use. As an example, we may consider the processes of shale oil production, coal liquefacation and coal gassification.

In general, it is felt that many of the final products, as well as process waters, may contain components even more harmful than in the petroleum analogues. Polynuclear aromatics and nitrogen heterocycles are classes of particular concern as possible carcinogens or mutagens. The Total Luminescence Spectrometer is designed to monitor these types of materials, both as complex mixtures and as chromatographically separated fractions. It should be noted that all of the modern luminescence techniques discussed earlier, including room temperature phosphorescence, synchronous scanning, laser excitation, etc., will be possible on the instrument. Thus it should be useful in extending current work as well as allowing new applications.

Compounds of interest include polynuclear aromatic hydrocarbons, nitrogen heterocycles, hydroaromatics, mono-and polyphenols. Because it is possible to obtain both fluorescence and phosphorescence at room temperature from TLS plates, filter paper etc., the instrument may be used for characterization, identification and quantitation of samples on solid surfaces.

Since chromatographic methods do not always separate all compounds completely, the instrument will be valuable as a specific and sensitive detector. The synchronous scan luminescence spectrometry employed by Vo-Dinh (1978) corresponds to a special "cut" in the total luminescence spectrum. The present instrument can be programmed to produce any desired synchronous scan spectrum, thus enhancing the utility of this concept.

While the instrument can perform completely analysis only on simple mixtures, it is also useful to analyze changes occurring in complex mixtures, such as in "weathering" of petroleum oil.

Nitrogen Heterocycle Mixture

To illustrate the applicability of the TLS concept to heterocycles, which are found in abundance in shale oil, we made up a mixture of 0.077 ppm carbazole, 0.069 ppm 13-H-dibenzo(a,i)carbazole, 0.4 ppm 2,3-dimethylindole, and 1.06 ppm 4-methyl quinoline (lepidine), all in methylcyclohexane. Data for contour spectra were obtained by serial scanning of a Baird Corporation Model FC-100 Spectrofluorometer equipped with a rhodamine B quantum counter. Sample and reference signals were digitized and entered directly into a PDP 8 Minicomputer. Spectral correction of data and contouring were performed on an IBM 1130. While these data were not acquired on the instrument discussed earlier, they illustrate the type of output to be expected.

Total luminescence contour spectra for the mixture and for the first three components are given in Fig. 3. While there is considerable overlap in the

spectra, the practiced eye can easily discern the presence of the three components in the mixture, due to the local symmetry. The 4-methyl quinoline (lepidine) is not visible at room temperature because it does not fluoresce; however analysis at 77^0K permits clear determination utilizing phosphorescence.

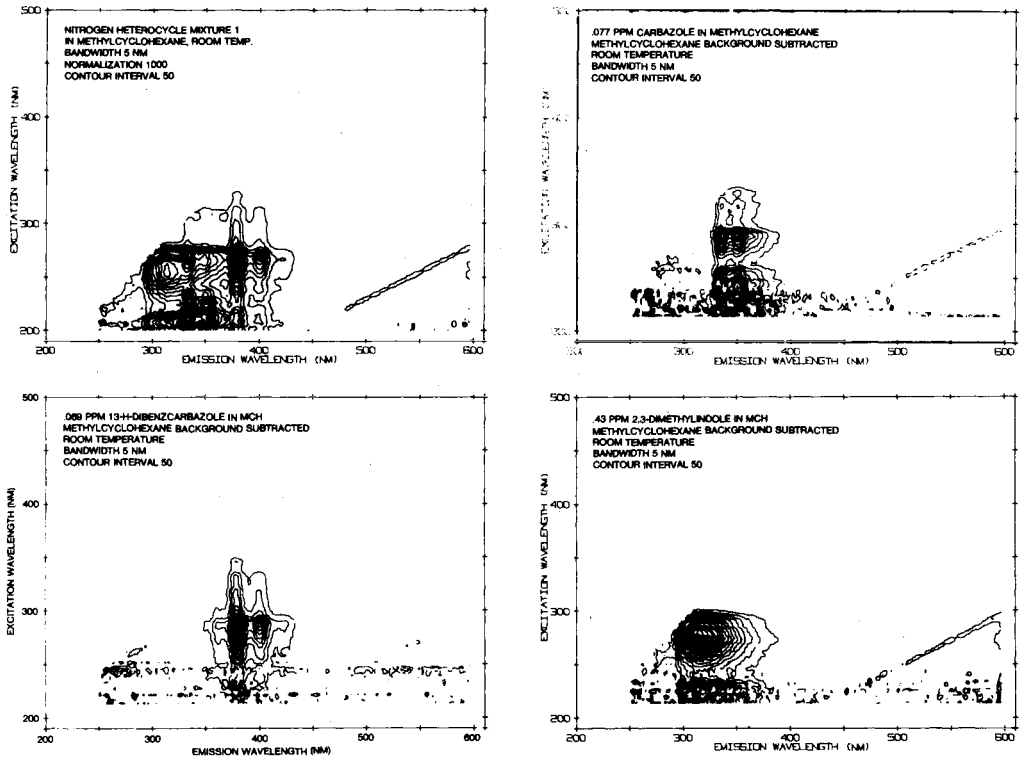

Fig. 3. Total luminescence contour spectra of nitrogen heterocycle mixture and fluorescent components.

CONCLUSIONS

The total luminescence spectrometer will allow full use of luminescence techniques for aromatic mixture analysis. The extreme speed of the system will allow direct coupling to chromatographic systems, and the versatile computer system will allow rapid analysis of data, including identification and quantitation. The instrument is expected to be particularly valuable in analyzing and monitoring toxic and hazardous materials in the environment.

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Analysis of C_6-C_{20} Hydrocarbons in Semi Rural Zones by High Resolution Gas Chromatography Coupled to Mass Spectrometry

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ABSTRACT

A technique of trace analysis on high resolution capillary columns is described. Qualitative and quantitative analysis of C_6-C_{20} hydrocarbons in ambient atmospheres of semi rural type are made. Different adsorbents have been tried in order to trap organic gases. The use of the Grob and Grob method for the enrichment of organic vapours on charcoal has succesfully been applied to a low volume sampling technique. High resolution glass capillary columns coupled to mass spectrometry enable qualitative analyses of more than sixty components in a single run. After identification, quantitative analysis could be performed by means of the co-chromatography of the sample with a calibrated standard mixture containing sixty organic compounds. Some limitations and efficiency problems of the methods are discussed.

KEY WORDS

Hydrocarbons in air. Charcoal trap. Glass capillary columns. GC-MS analysis.

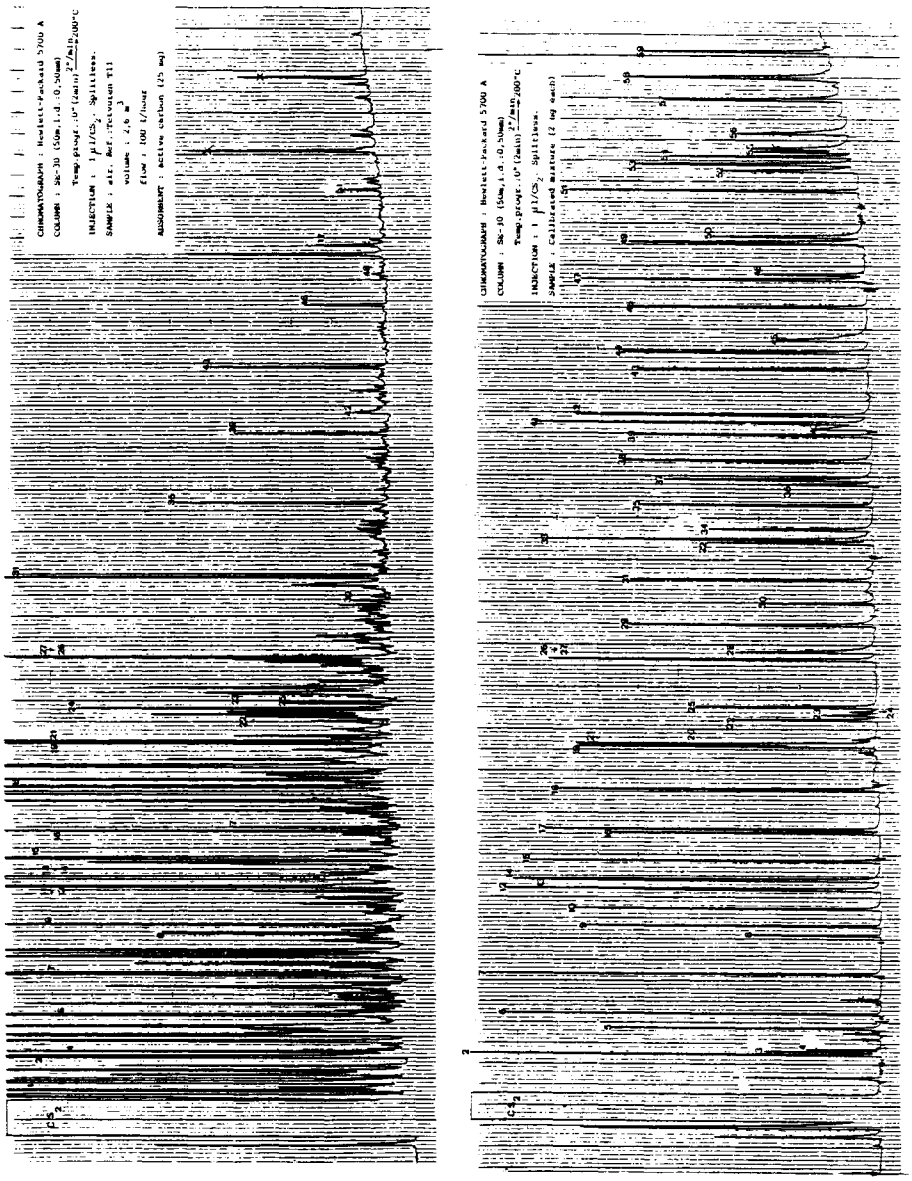
INTRODUCTION

In the course of our studies of the deleterious effects of atmospheric oxydants on plants, we discovered that the concentrations of "non methane hydrocarbons" often bad correlate with the ambient levels of ozone measured in rural and semi rural zones. Since then, we decided to investigate the nature of the "non methane hydrocarbon" fraction and to develop a method which could be sensitive enough to measure the trace level concentrations of the reactive hydrocarbons responsible for oxydant formation. Different trapping procedures have been tested, together with GC-MS analysis methods. We are now able to report the results we obtained, using the Grob and Grob (1971) method.

EXPERIMENTAL

Sampling.

The ambient air is pumped through the adsorbent, protected from dust pollution by means of a glass fiber filter (pore size : 0,3 to 10 µ).



Qualitative analysis of organic compounds in ambient air. Numbers refer to substances listed in tables I and II.

TABLE I CALIBRATED MIXTURE FOR SE-30 (50 m)

1. chloroforme
2. benzène
3. tétrachlorure de carbone
4. cyclohexane
5. trichloréthylène
6. n-heptane
7. toluène
8. tétrachloréthylène
9. n-octane
10. chlorobenzène
11. 1-chlorohexane
12. éthyltoluène
13. m-xylène
14. p-xylène
15. o-xylène
16. n-nonane
17. isopropylbenzène
18. 1,3,5-triméthylbenzène
19. 1,2,3-triméthylbenzène
20. n-décane
21. 1,2-dichlorobenzène
22. m-éthyltoluène
23. p-éthyltoluène
24. o-éthyltoluène
25. 1-chlorooctane
26. 1,3,5-trichlorobenzène
27. n-undécane
28. camphre
29. naphtalène
30. 1,2,3-trichlorobenzène
31. n-dodécane
32. 1-chlorodécane
33. 2-méthylnaphtalène
34. 1-méthylnaphtalène
35. n-tridécane
36. 1,2,3,4-tétrachlorobenzène
37. biphényl
38. 2,6-diméthylnaphtalène
39. n-tétradécane
40. diméthylphtalate
41. hexaméthylbenzène
42. acénaphtène
43. n-pentadécane
44. fluorène
45. diéthylphtalate
46. n-hexadécane
47. 1-méthylfluorène
48. hexachlorobenzène
49. phénanthrène
50. anthracène
51. n-octadécane
52. 2-méthylanthracène
53. 1-méthylanthracène
54. 1-chlorohexadécane
55. 9-méthylanthracène
56. dibutylphtalate
57. fluoranthène
58. pyrène
59. 1-chlorooctadécane

Numbers correspond to those in the figure.

TABLE II DETERMINATION OF C_6-C_{20} HYDROCARBONS IN AMBIENT AIR

SAMPLE : TERVUREN (T), Volume : 2,6 m^3 (3-16-1978)

Identified substances	Co-chromato-graphy	Concentration in air (ppb)	Identified substances	Co-chromato-graphy	Concentration in air (ppb)
1. Chloroforme	x	0,94	- Styrène		
2. Benzène	x		22. m-Ethyltoluène	x	
4. Cyclohexane	x		23. p-Ethyltoluène	x	0,02
6. n-Heptane	x	0,08	24. o-Ethyltoluène	x	
7. Toluène	x	0,62	- Méthylstyrène		
- iso-Octane			- iso-Undécane		
8. Tetrachloréthylène	x	0,005	27. n-Undécane	x	0,015
9. n-Octane	x	0,04	- iso-Dodecane		
- iso-Nonane			- iso-Dodecane		
- iso-Nonane			- sec-butylbenzène		
- Ethylbenzène	x	0,14	- tert.butylbenzène		
13. m-Xylène	x	0,45	31. n-Dodecane	x(St.Int)	0,016
14. p-Xylène	x		- iso-Tridécane		
15. o-Xylène	x	0,15	35. n-Tridécane	x	0,010
- iso-Nonane			39. n-Tetradécane	x	0,007
16. n-Nonane	x	0,03	- n-Pentadécane	x	0,008
- n-Propylbenzène			46. n-Hexadécane	x	0,002
17. iso propylbenzène	x	0,01	- n-Heptadécane		
18. 1,3,5-Triméthylbenzène	x	0,04	51. n-Octadécane	x	0,002
19. 1,2,3-Triméthylbenzène	x	0,03			
- iso-Decane	x	0,02			
20. n-Decane					

Numbers correspond to those in the figure.

The adsorbents we tested are TENAX GC, CHROMOSORB 102, PORAPAK Q, SPHEROSIL XOA 400 and a treated charcoal. The home-made filters contain 100 mg of polymeric adsorbent or 25 mg of charcoal. The charcoal filters have been prepared according to Grob and Grob (1971).

Elution

The samples are thermically eluted (250°C) from the polymeric adsorbents during five minutes and then directly injected in front of the capillary column which is kept at low temperature (0°C) until elution is accomplished. The normal program of temperature is then started. The samples adsorbed on charcoal are eluted with 100 µl carbon disulfide, as described by Grob and Grob (1976).

Analysis

The gas chromatographs (Carlo Erba 2900, Hewlett-Packard 5700 A and Varian 2700) are equiped with WCOT glass capillary columns which possess the required performance to perform trace analyses of ambient hydrocarbons. Three different columns have been used for this work : Ucon HB 5100 (20 m, i.d. : 0,32 mm); SE - 30 (50 m, i.d. : 0,50 mm) and OV-1 (20 m, i.d. : 0,32 mm). The Varian gas chromatograph, equiped with the SE-30 column, is coupled with a Varian Mat 311 A mass spectrometer.

RESULTS AND DISCUSSION

The use of porous polymers is delicate since it is difficult to obtain the acceptable blanks required for high resolution analyses. Even the method of conditioning described by Versino (1974) is often impracticable. Nevertheless, we determined the "breakthrough volume" of a polymer load of 100 mg for different substances. Air was pumped through the filter, at room temperature, with a rate of approximately 100 l/hour. This study establishes that the adsorbing efficiency of C_6-C_{20} hydrocarbons is greater than 95% only when the volume of sampled air is smaller than 3 l.
With the Grob charcoal filters, it is possible to sample up to 250 l of air without appreciable loss of hydrocarbons. This has been measured as we let air flow through two identical charcoal traps, disposed in series. The total efficiency of the method (when correcting for losses of the first trap, measured on the second trap) is greater than 95% for C_6-C_{20} hydrocarbons when 2500 l of air are sampled at a flow rate of 100 l/hour. The figure shows a typical gas chromatogram obtained in these conditions, together with a chromatogram of the calibrated standard mixture used for quantitation of the organic compounds. The numbers refer to the hydrocarbons listed in table I and table II. Table II gives an example of a GC-MS quantitative analysis of ambient air of a semi rural zone.
Until now, we sampled air only from semirural regions where background levels (30 to 50 ppb) of ozone have been measured. In these zones, the ubiquity of alkylbenzenes (probably of man-made origin) is remarkable. We are now looking forward for the presence of "reactive" natural hydrocarbons by sampling air in regions of dense vegetation.

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Quantitative Analysis of Methylchloride in the Atmosphere by Gas Chromatography with Electron Capture Detection

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ABSTRACT

The relative importance of natural and anthropogenic sources of halogenoalkanes can be determined from the quantitation of CH_3Cl. The sensitivity of the electron capture, detector has been determined in the two modes of operation, the fixed frequency sampling mode and the constant current modulated frequency. The sensitivity is 40 times larger in the first case. The electron capture detector has been calibrated, using the permeation tube technique. The limit of detection for methyl chloride is 0.1 ppb in a 5 ml sample. A trapping preconcentration procedure can increase the size of the air sample analyzed and reduce the detection limit to 10 ppt.

INTRODUCTION

The chlorofluorocarbons which are found at a measurable level in the atmosphere are suspected to participate to a mechanism of ozone destruction (Molina and Rowland, 1974) - Lovelock (1974)has shown however, that it is necessary to determine the relative contributions to the stratospheric concentration of haloalkanes of the anthropogenic sources and of the natural ones. With the electron capture detector (ECD) methylchloride one of the most important chlorocarbons of natural origin is quite difficult to detect at low trace levels (Lovelock, 1975 ; Cox et al, 1976 ; Grimsrud and Miller, 1978) and the GC-MS technique has been widely used for its quantitative analysis (Grimsrud and Rasmussen, 1975; Cronn and Harsch, 1976 ; Cronn et al, 1977 ; Russel and Shadoff, 1977). Grimsrud and Rasmussen (1975) have reported that the relatively large concentration in the troposphere of the naturally occuring CH_3Cl is $530 \pm 30 \times 10^{-12}$ ppv . Their measurements indicate that chlorofluorocarbons constitute only 36% of the total organic chloride in the atmosphere. Cronn et al (1977) have found that the halocarbon with the largest tropospheric concentration

is CH_3Cl with 569 x 10^{-12} ppv. The analysis technique used is sample collection followed by laboratory analysis using GC/MS as described by Cronn and Harsch (1976). The above techniques necessitate expensive instrumentation and do not permit on-field measurements as does the ECD. Lovelock (1975) has shown however that the sensity of electron capture detection of CH_3Cl is markedly improved if the detector is used at high temperature. The limit of detection for a 5 ml sample (direct injection) is 8.5 x 10^{-11} (v/v). Cox et al (1976) have measured the concentrations of CH_3Cl and CH_2Cl_2 in air using an ECD and a hundred fold preconcentration of the air sample by condensation at -196°C. Concentrations of 690 x 10^{-12} ppv for CH_3Cl and 35 x 10^{-12} ppv for CH_2Cl_2 have been measured at ground level with standard deviations of 390 x 10^{-12} ppv and 19 x 10^{-12} ppv respectively. In this work we shall report a detailed study of the quantitative analysis of CH_3Cl using the fixed frequency pulse sampling mode for the ECD and show that the detection limit of a given detector cell is lower than with the constant-current pulse modulated detection mode.

EXPERIMENTAL

The experiments were carried out on a Varian 3700 Aerograph gas chromatograph which incorporates an automatic Valco 8-port injection valve purged with carrier gas and an ECD operated at 280°C throughout this study. The carrier gas was high purity nitrogen (Air Liquide, Paris, France) with O_2 and H_2O < 5 ppm. The carrier gas was first passed through an activated charcoal filter. The flow rate is 40 ml/min. The Varian cell with its 8mCi Ni ^{63} radioactive source (Patterson, 1977) was used but instead of the instrument electronics (constant-current, variable frequency of constant voltage pulses) we used a simpler electronic system with constant frequency constant voltage pulses, provided by J.E. Lovelock. The pulse period is 250 µsec, the pulse width 2.6 µsec and the pulse amplitude 23 Volts. The anode is connected to the electronic system which incorporates the pulse generator and the amplifier; the cathode (radioactive foil) is connected to the ground. A comparison between the performances of the two systems was made at the beginning of the study. The constant period system was found more sensitive than the constant current one because of a lower noise level and was used throughout all the analytical study. It was later found that the above pulse specifications are close to the best operating conditions of the detector cell as determined by connecting the cell to a Hewlett-Packard pulse generator and a Keithley electrometer changing the pulse period, width and height and making injections of a constant composition gas flow. The 2-meter long column is packed with Carbopack C-HT (Supelco, Bellefonte, U.S.A). This adsorbent (surface area 12 m^2/g) is a thermal carbon black graphitized at 3000°C and purified by heating at 1000°C under an hydrogen stream. For calibrating the instrument for CH_3Cl, we have used the permeation technique described by Scaringelli et al (1970). The permeation tube is a microbottle and CH_3Cl permeates through a small teflon septum of 3 mm^2 surface and 2 mm thickness. The permeation rate for CH_3Cl is 2.5 x 10^{-9} g/min at 20°C, determined by gravimetry over a period of a few

months. The permeation tube is placed in a stream of pure nitrogen whose flow rate is adjusted to obtain the required concentration. By this method a whole range of concentration between 10^{-10} to 10^{-7} ppv can be achieved. The flow of calibrated gas is passing continuously through a 1.5 ml sampling loop which can be introduced in the carrier gas flow when needed.

RESULTS

1. Qualitative analysis

It has been shown that graphitized carbon black, either uncoated (gas solid chromatography, Vidal-Madjar et al 1978) or modified with small amounts of stationary liquid phases (gas solid-liquid chromatography (Bruner et al, 1978), can separate with good selectivity the main halogenoalkanes. Because of the high sensitivity which is aimed at, gas solid chromatography has bean preferred for this work. Moreover a better resolution is obtained between $C_2H_3Cl_3$ and CCl_4.

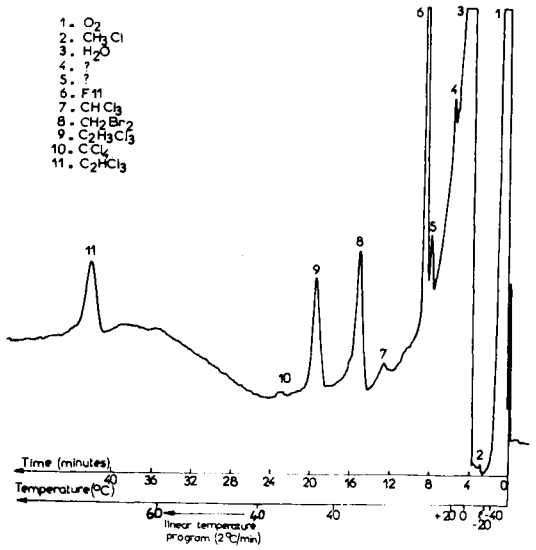

Figure 1 - Analysis of atmospheric air sample on graphitized thermal carbon black. Sample loop : 1.5 cm^3 - nitrogen flow-rate : 40 ml/min. Fixed frequency ECD mode Detector temperature 280°C.
Standing current I_o = 1.5 x 10^{-9} A; full scale deviation = 1.8 x 10^{-10} A.

Figure 1 shows the analysis of a 1.5 ml sample of out door air which is quite polluted in Freon 11, probably because of the proximity of the laboratory building. CH_3Cl is well separated from oxygen and water ; the amount detected is 0.5×10^{-9} ppv close to the sensitivity limit of our instrument for the 1.5 ml sample loop used. A separation like the one shown on Figure 1 cannot be obtained by isothermal analysis. The temperature of the column is programmed from - 55°C to 60°C. The column is placed inside a teflon tube cooled by connecting it to a cylinder of CO_2. This cooling device keeps the column at - 55°C until injection when programming of the oven temperature begins. The same technique is used for larger samples or when trapped samples are analyzed.

2. Quantitative analysis of CH_3Cl

For an ECD operated with the pulse sampling mode Wentworth et al (1966) have demonstrated that the following relationship is valid

$$\frac{I_o - I}{I} = K C \qquad (1)$$

where K is the capture coefficient, I_o the standing current and I the detector current in the presence of a capturing species at concentration C. For small values of $\Delta I/I_o$, with a relative error equal to $\Delta I/I_o$ we can write :

$$I_o - I = \Delta I = I_o K C \qquad (2)$$

The detector behaves as a concentration sensitive sensor for CH_3Cl, the concentration at peak maximum is (Guiochon, 1964) :

$$C_{max} = \cdot \frac{m \sqrt{N}}{\sqrt{2\pi}\, V_R^{\circ}} \qquad (3)$$

where m is the amount injected, N the number of theoretical plates of the column and V_R° the retention volume. A plot of $(I_o-I)_{max}$ is thus a linear function of the amount injected m as long as the ratio $(I_o-I)_{max}/I$ is small. The instrument calibration curve is given in Figure 2 where ΔI_{max} is plotted as a function of the amount injected expressed in ppv at s.t.p. (2.25 pg/cm^3 for 1 x 10^{-9} ppv). A straight line is obtained which passes through the origin. For this calibration curve, theoretical deviation from linearity is not larger than 2% for the largest amount of sample injected as the standing current is $I_o = 1.5 \times 10^{-9}$ A.

In order to obtain the best response for CH_3Cl the electron capture detector was operated at 280°C. The temperature dependance of the response is obtained by plotting ln $KT^{3/2}$ or ln $(\Delta I/I)T^{3/2}$ versus 1/T. The negative slope of 14 kcal/mole is in good agreement with the results of Wentworth and Chen (1967). It is indicative of a dissociative electron attachment mechanism (Wentworth et al, 1966) and can be used for the characterization of CH_3Cl (Cox et al 1976). It demonstrates the necessity to keep the detector cell at high temperature as a 10-fold decrease in sensitivity is observed when temperature is decreased from 280 to 200°C. For a precise quantitative analysis of CH_3Cl (± 1%) the detector cell temperature has to be controlled within ± 0.5°C.

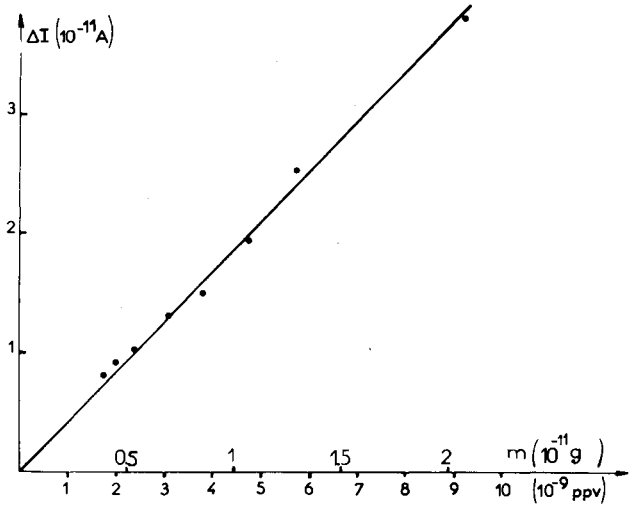

Figure 2 - Instrument calibration curve of CH_3Cl for a 1 ml sample loop. Fixed frequency ECD mode. Detector temperature : 280°C. Standing current I_o = 1.5 x 10^{-9} A.

Minimum detectable amount of CH_3Cl

With a noise level of 1 x 10^{-12} A, the minimum detectable amount of CH_3Cl which gives a signal equal to twice the noise level is 1 x 10^{-12}g which corresponds to 0.1 x 10^{-9} ppv for a 5 ml sample loop. This is the largest admissible air volume in order to have precise measurements in quantitative analysis. Figure 3 shows the chromatogram of a nitrogen sample containing 5 x 10^{-12}g CH_3Cl Equation (3) gives a minimum detector response of 0.2 x 10^{-12}g/cm^3 with m = 1 x 10^{-12}g, N = 1020, $\overline{V_R}°$ = 68 cm^3, which is in satisfactory agreement with the result of Figure 3.

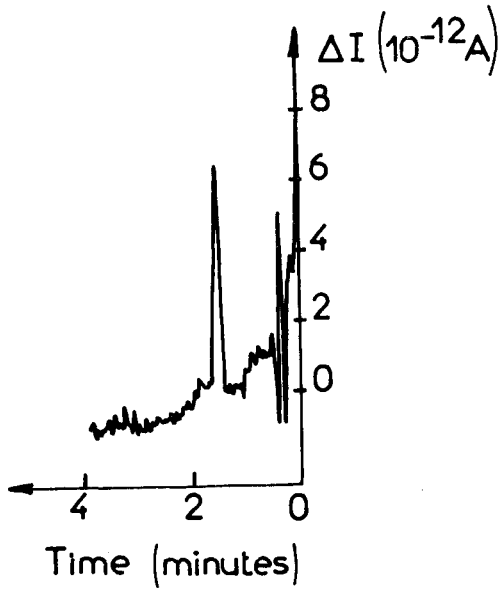

Figure 3 - Elution peak of CH_3Cl
amount injected : 5 x 10^{-12}g. Fixed frequency ECD mode
nitrogen flow rate : 40 ml/min. Column temperature 25°C
detector temperature 280°C

Figure 4 compares the signal for CH_3Cl using the original Varian electronics (constant current pulse modulated mode) and the system used throughout this work. Both analyses are run with the same experimental conditions and the amount of CH_3Cl injected is 1 x 10^{-10}g. In the first case the detection limit is 0.4 x 10^{-10}g A decrease of this limit by a factor 40 is achieved when operating in the fixed frequency mode, with optimized parameters. The constant current, variable frequency mode of operation has been widely adopted because of its larger linear dynamic range. However, Lovelock and Watson (1978) have shown recentely that its response with compounds that strongly attach electrons is very non-linear. It thus appears that the fixed frequency mode of operation is not without some advantages. It has to be preferred for accurate analysis of halogenoalkanes : well-known response function is

obtained as well as an increased sensitivity for weakly absorbing species which is caused by a better signal on noise ratio and can be controlled by changing easily the operational parameters of the ECD.

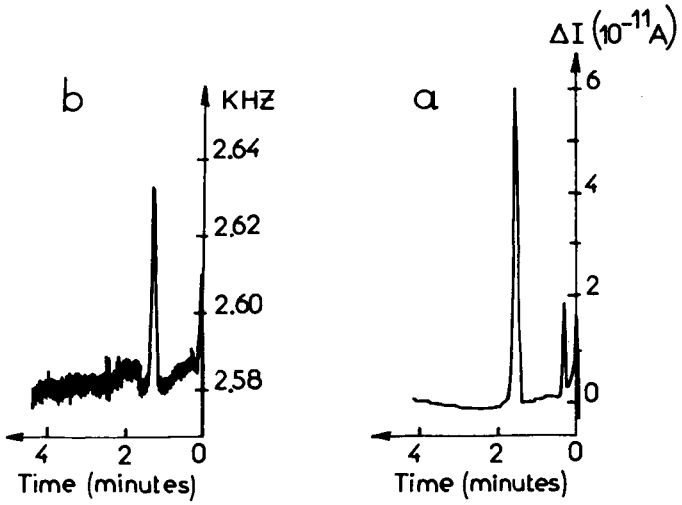

Figure 4 - Comparison of the ECD response of CH_3Cl with the fixed frequency mode (a) and the constant current modulated pulse sampling mode (b)
amount injected : 1 x 10^{-10} g
nitrogen flow rate : 40 ml/min. Column temperature 25°C
detector temperature 280°C

Trapping procedure for CH_3Cl

To decrease the detection limit it is possible to inject larger sample amounts, using air preconcentration techniques (Cox et al, 1976 ; Cronn et al, 1977) or trapping procedures on inert adsorbents (Russel and Shadoff, 1977 ; Bruner et al, 1978). The last technique is more advantageous as oxygen is almost eliminated from the sample. The maximum sample volume (MSV) of air derived from retention volume measurement (Vidal-Madjar et al, 1978) when using a 10 cm long 4 mm i.d trap of Porapak T is given in Table 1 for various chlorofluoro carbons.

TABLE I

Maximum sample volume (MSV) of air with a Porapak T 10 cm long, 4 mm i.d. Trap at 20°C.

Solute	MSV (cm^3)
$CClF_3$	19
CH_3Cl	69
CCl_2F_2	63
CH_2Cl_2	3,900
CCl_3F	370
$CHCl_3$	8,200
$C_2H_3Cl_3$	8,100
CCl_4	5,900
C_2HCl_3	15,000

A 60 ml sample of air can be analyzed with complete trapping of CH_3Cl at ambient temperature. The minimum detectable amount for CH_3Cl is then 10^{-11}ppv which permits a precise quantitative analysis of CH_3Cl in the atmosphere. Analysis is carried out by replacing the sample loop of the gas sampling valve by a trap and heating it at 200°C.

CONCLUSION

This work shows that the use of the fixed frequency mode permits an increase in the sensitivity of the ECD detection of the weak capturing species like CH_3Cl. It is not necessary to add oxygen to the nitrogen carrier gas (Grimsrud and Miller, 1978) to increase the ECD sensitivity , a procedure which might result in oxidization of the stationary phase or of the solutes to be analyzed. Quantitative analysis of the strong capturing species like CCl_3F and CCl_4, which is an important environmental problem (Lovelock and Watson, 1978) is under study using the permeation tube technique. This necessitates a high precision recording microbalance to achieve reliable standards in the 10^{-11} to 10^{-9} ppv range.

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A Recent Case of Environmental Pollution: Some Analytical Applications

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ABSTRACT

During recent years, the pollution problem has become ever more serious and many laboratories throughout the world are setting up particularly specific and sensitive methods for identifying traces of substances present in the environment. Recently in Northern Italy a serious accident occurred which polluted a densely populated area with an extremely toxic compound, identified as 2,3,7,8-tetrachlorodibenzo-para-dioxin (2,3,7,8-TCDD). In this paper the way in which this problem was tackled will be discussed. To monitor the degree of exposure of animals present in the contaminated areas, a method for routine identification and quantitative determination of 2,3,7,8-tetrachlorodibenzo-para-dioxin has been developed.

KEYWORDS

Environmental pollution, 2,3,7,8-tetrachlorodibenzo-para-dioxin, gas chromatography, mass spectrometry, mass fragmentography.

The explosion on July 10, 1976, at a chemical factory in Seveso belonging to a Swiss multinational group, released a cloud of vapour which contaminated the surrounding area (Hay, 1976). The vapour, a chemical cocktail consisting primarily of 2,4,5-trichlorophenol, also contained an extremely toxic by-product, 2,3,7,8-tetrachlorodibenzo-para-dioxin, which has twenty two possible positional isomers depending on the position of the chlorine atoms.
The explosion occurred six and a half hours after the weekend shut-down: in the reactor producing 2,4,5-trichlorophenol the reaction became exothermic and reached the critical limit, blowing the safety valve and discharging the hot vapour directly into the atmosphere. When the solvents evaporated, 2,4,5-trichlorophenol crystals and 2-3 kg of 2,3,7,8-tetrachlorodibenzo-para-dioxin in the form of dust were precipitated south of the factory, over an area inhabited by several thousand people. Local police were informed of the explosion on the Saturday it occurred, the mayor of Seveso on the Sunday, and the local authorities in adjoining townships on the Monday: they banned the consumption of fruit and other crops from around the factory. A few days later, cases of dermatitis and gastrointestinal symptomatology appeared, mainly among children, and domestic animals started to die. Only after two weeks 2,3,7,8-tetrachlorodibenzo-para-dioxin was first identified by the Milan Provincial Laboratory of Hygiene and Prophylaxis and confirmed by the Swiss multinational group.

Only then did the authorities realize the crisis and evacuate the contaminated area, having hitherto been told by the chemical factory that the worst component of the vapour was 2,4,5-trichlorophenol; it is this delay that caused concern, particularly in view of the known toxicity of 2,3,7,8-tetrachlorodibenzo-para-dioxin.
Similar accidents had occurred in Germany (in 1953) and other countries, but without putting populations at risk (Hay, 1976). In the Italian factory, located in a built-up area, the safety valve was located in a vent pipe leading to the atmosphere, rather than into the factory.

Despite the formidable situation, the authorities of the Lombardy Region (where Seveso is located) took a scientific approach. The Istituto Superiore di Sanità in Rome, the Milan Provincial Laboratory of Hygiene and Prophylaxis, the University of Milan Institute of Pharmacology and Pharmacognosy, and our own Institute cooperated in mapping out the polluted zones, firstly with $\sim$ 1000 assays for 2,3,7,8-tetrachlorodibenzo-para-dioxin in soil and plants by gas chromatography and mass spectrometry. Such a sensitive method was necessary to enable even traces of the highly toxic product to be revealed: in an intermediate zone ('B') 2,3,7,8-tetrachlorodibenzo-para-dioxin was scattered at levels between 0.75 and 50 $\mu g/m^2$.
The medical and other investigations performed, e.g. of animal death patterns and 2,3,7,8-tetrachlorodibenzo-para-dioxin levels in the liver of surviving animals, and the remedies taken are hardly whithin the scope of the present account of analytical aspects.

2,3,7,8-tetrachlorodibenzo-para-dioxin is produced as a by-product during drastic alkaline hydrolysis of tetrachlorobenzene to form 2,4,5-trichlorophenol. This hydrolisis, if not very carefully controlled, favours the formation of 2,3,7,8-tetrachlorodibenzo-para-dioxin, the most common chlorodibenzo-para-dioxin present in 2,4,5-trichlorophenoxyacetic acid (Hay, 1976). Other toxic polychlorodibenzo-para-dioxins are also produced, theoretically at least 75, having 1 to 8 chlorine atoms attached to the two benzene rings. At least ten have been synthesized, and one or two more have been found in food, animal feeds and 2,4,5-trichlorophenoxyacetic acid.

Like other chlorodioxins, 2,3,7,8-tetrachlorodibenzo-para-dioxin is stable to heat, acids and alkalis; it is inactivated only at over 800° or by UV light. It is virtually insoluble in water (0.0002 ppm), only slightly soluble in fats (44 ppm in lard oil), and more soluble in hydrocarbons (570 ppm in benzene), and most soluble in chlorinated organic solvents (1400 ppm in 0-dichlorobenzene). 2,3,7,8-tetrachlorodibenzo-para-dioxin is also relatively immobile in soil; it moves slowly from the surface into the subsoil, but is not taken up by plants (Hay, 1976). Living organisms metabolize it only at a very slow rate, if any, and they excrete it extremely slowly. For example, in the rat it takes about three weeks for body levels of 2,3,7,8-tetrachlorodibenzo-para-dioxin to fall by 50%.

2,3,7,8-tetrachlorodibenzo-para-dioxin is one of the most toxic compounds known, with $LD_{50} \sim 1$, ~ 20 and ~ 100 $\mu g/Kg$ in the guinea pig, rat and rabbit respectively. Indirect observations on man (including Seveso) suggest an acute toxicity no higher than for the guinea pig. It is embryotoxic and teratogenic, strongly immunosuppressive especially in newborns, and also it is hepatotoxic although the mechanism is unclear (Garattini, 1977). It enhances the enzymatic activity of the endoplasmic reticulum, thus probably altering liver function and leading to massive degeneration of the hepatocytes; since this is the site

where toxic intermediates are formed from carcinogens, 2,3,7,8-tetrachlorodibenzo-para-dioxin may increase the carcinogenicity of some chemicals and the immunosuppressive effect may aggravate matters. There is still some disagreement on whether 2,3,7,8-tetrachlorodibenzo-para-dioxin is mutagenic in bacteria at very high concentrations.

In a first phase our efforts were devoted to the identification of 2,3,7,8-tetrachlorodibenzo-para-dioxin in the numerous samples of tissues of dead animals, which were collected by the Regional Veterinary Service and stored for analysis.
It is reported in the literature that 2,3,7,8-tetrachlorodibenzo-para-dioxin preferentially accumulates in liver and fat of exposed animals; therefore we started looking for 2,3,7,8-tetrachlorodibenzo-para-dioxin in these sites.
The extraction procedure, adapted from Baughman and Meselson (1973), was as follows. Liver samples (7-8 g) weighed and homogenized in 2 vol. of absolute ethanol, were saponified with a further 3 vol. of 10 N NaOH for one hour at 100 °. The mixture was extracted with n-hexane (3 x 20 ml) for 15 min periods on an automatic shaker. The organic phase, carefully evaporated down to 5 ml under nitrogen, was washed four times with sulphuric acid and then once with water, with shaking for 10 min each time. The sample was neutralized by filtration through a column of sodium carbonate, equilibrated beforehand with n-hexane. 2,3,7,8-tetrachlorodibenzo-para-dioxin was eluted with 10 ml of n-hexane. The eluate, taken to dryness under nitrogen at 60 °, was re-dissolved in n-hexane and, for the identification, injected into a gas chromatographic-mass spectrometric apparatus. This was a Finnigan 3100 instrument, equipped with a data system Finnigan 6000 or an LKB 9000 gas cromatograph-mass spectrometer.

At the outset of the program we found, in rabbit liver (Abbruzzi and co-workers, 1978), high levels of 2,3,7,8-tetrachlorodibenzo-para-dioxin that enabled us to obtain the complete mass spectrum of 2,3,7,8-tetrachlorodibenzo-para-dioxin, giving further confirmation of identity. The mass spectrum of 2,3,7,8-tetrachlorodibenzo-para-dioxin is relatively simple. The base peak is the molecular ion at m/e 320. As a result of the various possible combinations of the naturally occurring ^{35}Cl and ^{37}Cl isotopes in a tetrachloro compound, the signal for the molecular ion is a pentuplet with peaks at m/e 320, 322, 324, 326 and 328 with intensities in the ratio 78 : 100 : 49 : 10 : 1.

2,3,7,8-tetrachlorodibenzo-para-dioxin was found in many other animals samples, its identity confirmed by mass spectrometry linked to high resolution glass capillary columns, OV 17 (25 m) and OV 101 (27 m), in cooperation with the Swiss Federal Research Station for Agriculture, Horticulture and Arboriculture, in Wadenswil, Switzerland.

The second phase of the program (Abbruzzi and co-workers, 1978) was aimed at measuring 2,3,7,8-tetrachlorodibenzo-para-dioxin routinely in tissues of different animal species. Mass fragmentography was adopted as giving the requisite specificity and sensitivity. The instrument was a gas cromatograph-mass spectrometer LKB 2091, equipped with a computer system LKB 2130 for data acquisition and automatic calculation of results.

A 2 m glass column, 4 mm i.d., packed with 3% OV 1 on Gas Chrom Q (100-120 mesh) was run at 250 °, and the ion source was used in the electron impact mode with an electron energy of 70 eV. For mass fragmentography the instrument was focussed on the ions at m/e 320 and 322. For quantitative determina-

tion 2,3,7,8-tetrachlorodibenzo-para-dioxin labelled with ^{37}Cl was used as an internal standard, and was detected by monitoring the molecular ion at 328.

Sensitivity and linearity of the gas chromatographic-mass fragmentographic method were tested by injecting a series of solutions containing various concentrations of 2,3,7,8-tetrachlorodibenzo-para-dioxin and a constant amount of ^{37}Cl-2,3,7,8-tetrachlorodibenzo-para-dioxin. The minimum instrumental detectable amount was 10 pg, with a signal-to-noise ratio better than three on the two focussed ion. A linear response was obtained over the load range 10-500 pg (Reid, 1978).

For sample extraction and clean-up, the above procedure was applied; the overall recovery was 65% and the minimum detectable amount 2.5 ng/g of liver. Unfortunately the presence of interfering compounds precluded 2,3,7,8-tetrachlorodibenzo-para-dioxin measurement in several samples. It was therefore decided to improve the specificity of the extraction and clean-up procedure and, after several attempts, a compromise was reached between sensitivity, sample clean-up and throughput. The overall recovery is ~75% and 2,3,7,8-tetrachlorodibenzo-para-dioxin concentrations as low as 250 pg/g of liver can be routinely measured.

2,3,7,8-tetrachlorodibenzo-para-dioxin is identified on the basis of its gas chromatographic retention time (3.5 min) and the presence of a correct isotopic ratio between the two focussed ions at m/e 320 and 322 (78%).

To establish the variability and the confidence limits of the 320 : 322 ratio, statistical analysis was made on the isotopic ratio obtained injecting standard solutions of 2,3,7,8-tetrachlorodibenzo-para-dioxin and samples obtained by adding known amounts of 2,3,7,8-tetrachlorodibenzo-para-dioxin to the liver and carrying out the whole extraction procedure. The standard and the latter each showed (by the Lilliefors test) a normal distribution, the mean ratios being 78.3 and 76.2 with fiducial limits (P = 95%) 78.3 ± 0.84 and 76.2 ± 3.99; with the Welch test the two means did not differ.

The mass fragmentographic peaks at m/e 320 and 322 are obtained only with 2,3,-7,8-tetrachlorodibenzo-para-dioxin standard and not with 2,4,5-trichlorophenol standard. This means that 2,3,7,8-tetrachlorodibenzo-para-dioxin cannot be produced as an artefact, under our conditions of analysis, from 2,4,5-trichlorophenol by thermal degradation. A further study was performed (Abbruzzi and coworkers, 1978) to demonstrate chat the peaks cannot be produced by a 'pre-dioxin'.

The gas chromatographic-mass fragmentographic method has already been applied for the analysis of many liver samples of different animal species from the Seveso area. The findings were tipically positive with rabbits and for the few goats and hares examined, but not for the few domestic birds examined. In the goats (Abbruzzi and co-workers, 1978) which died in the vicinity of the factory, the levels were notably high (~1 µg/g), about ten times those prevalent in those rabbits that gave positive findings.

Interpretation of the 2,3,7,8-tetrachlorodibenzo-para-dioxin distribution amongst rabbits is not yet possible, because data on 2,3,7,8-tetrachlorodibenzo-para-dioxin kinetics in the rabbit are lacking in the literature and await a controlled-environment study which has now been started and which may illuminate the role of the time of exposure, dose and tissue concentration of

2,3,7,8-tetrachlorodibenzo-para-dioxin in determining toxicity. Kinetic data may also help interpret 2,3,7,8-tetrachlorodibenzo-para-dioxin levels in the organs of rabbits let loose in contaminated areas as a means of estimating spontaneous or artificially achieved reduction of environmental pollution levels.

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Evaluation of Organochlorine Compounds in the Emissions of Urban Incinerators

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ABSTRACT

The organochlorine compounds (pesticides, polychlorinated biphenyls and dioxins) because of their toxic properties represent a serious environmental hazard, so that the determination in urban incinerator emissions is a problem of great interest. The incinerator emissions include stack smoke, particulate matter recovered in the removal systems (electrofilter or water cyclone) and ashes.

The samples, after extraction and purification, are analyzed by gas chromatography (ECD) to indetify and evaluate the individual organochlorine constituents. The procedure has been applied to samples from emissions of urban incinerator operating either by direct combustion of by biochemical treatment of the waste. Evidence is given of the formation of dioxins during the incineration process.

INTRODUCTION

The recent accident occurred in a production plant of 2,4,5-trichlorophenol, in Seveso (Italy), which produced a large contamination of the sorrounding area by 2,3,7,8 -tetrachlorodibenzodioxin (TCDD), has stirred a great deal of interest in the establishment of new and/or reinforcement of existing regulations concerning industrial emissions. Quite recently, it has been shown by Olie and cow. (1977) that chlorodibenzo-p-dioxins (PCDD) and chlorodibenzufurans (PCDF) might be found as trace components of fly ash and flue gas of some municipal incinerators and their results have been confirmed by other investigators (Rappe, 1978; Busser and Bosshardt, 1978).

The problem of establishing the content of toxic and bioaccumulative pollutants is by far of a great importance for the definition of the most suitable procedures for urban waste treatment. Up to now, the efficiency of urban incinerators has been established in terms of concentrations of conventional pollutants (SO_2, NO_x, HC, HCl and particulated matter) but it seems that the determination

of the content of toxic compounds might be a more meaningful index.

It is well know that urban wastes contain considerable amounts of polychlorinated biphenyls (PCB) as well as pesticides and chlorophenols and that a certain amounts of these substances can be found in the emissions. The impact that these emissions might exert upon the environment has not been established, being the corresponding estimates affected by a variety of factors. The quality of the input material plays a determining influence but the type of the incinerator, its construction, the combustion temperature and the contact time in the gas phase also have an important role.

Although it has been established that chlorophenols and related products can be considered precusors of PCDD's, as laboratory experiments have shown (Ahlin and lindskog, 1977; Rappe and Marklund, 1978), no definite conclusions have been reached upon their formation from the incineration of ordinary city garbage. Furthermore, no attempt has been made to evaluate the impact that the incinerator's emission might have upon the environment in regards to their content in chlorinated organic compounds.

The evaluation of pesticides and PCBs in incinerators fly ash has been previously reported (Ashing and Lindskog, 1977). This study attempted to obtain information on the presence and concentration of PCDD in the incinerator emissions. By incinerator emissions we intend to refer to any material either in the condensed or in the gaseous phase arising from the combustion process and consequently the following classes of materials that must be examined:

a) ashes: any solid material obtained as a final product from the incineration process;
b) fly ash: coming from the abatement system used in the incineration process. It can be dusty material if an electrostatic precipitator is used or a mud if a cyclon with a water spray is used;
c) particulated matter in the fumes: corresponding to the material isokinetically sampled in the stack;
d) organic vapours in the fumes: material that can be trapped either by water condensation obtained by cooling the stack fumes, sampled by a probe or by absorption of the cooled fumes in an organic solvent.

EXPERIMENTAL

In order to obtain representative samples namely for the collection of the above c) and d) materials, the sampling system shown if fig. 1 has been used.

The particulated matter in the stack is sampled in isokinetic conditions into a steel vessel taped with quartz wool. Fume temperature after water condensation is about 15ºC. The trapping of the organic vapours is obtained by bubbling in toluene; by a needle valve a gas flow of 2 l/min. is realized, corresponding to about 1/10 of the total flow.

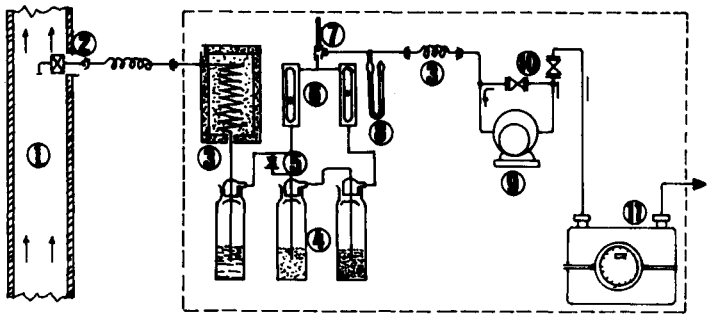

Fig. 1. Scheme of a stack sampler for an urban incinerator. 1-Stack, 2-Probe for particulate collector, 3-Condenser, 4-Impingers with toluene, 5-Needle valve, 6-Flow meters, 7-Thermometer, 8-Mercury manometer, 9-Vacuum pump, 10-Flow regulators, 11-Volume meter.

Direct G.C.- analysis of the chlorinated compounds

The various samples have been analyzed according to the following procedure: 10 g of the solid material (ash and fly ash or mud) or 1-2 g of particulated matter coming from the sampling of the fumes have been homogenized and dried at 60°C for 8 hours. Extraction was carried out in a soxhlet apparatus with benzene for 4 hours and the extract, after concentration to 1 ml under a nitrogen flow at about 50°C in a water bath, has been analyzed by gas chromatography.

The water condensate was extracted several times with small portions of benzene and the total benzene extract was finally evaporated and brought to 1 ml volume.

The toluene used as absorbing liquid in the two final traps of the sampling apparatus was also evaporated to 1 ml.

The gas chromatographic analysis has been made by using an electron capture detector with Ni 63 and by employing a 3 m glass column 2mm i.d. packed with Supelcoport 100-120 mesh + 1.5% SP 2250 + 1.95% SP 2401 at the following working conditions: carrier gas nitrogen: 3.5 atm.; flow: 30 ml/min.; temp. injector: 250°C.; temp. detector: 250°C.

All analyses have been performed with a standardized procedure so that the reported gas chromatograms are comparable. After the sample injection the column was operated for 8 minutes at 180° C and then temperature programmed (2.5°C/min) up to 245°C.

The peaks have been numbered and their identification as PCB and PCDD has been carried out by analyzing, in the same experimental conditions, standard mixtures of Arochlor 1248 and 1254 and various PCDDs (tetra, hexa, octachlorodioxin). Penta and heptachlorodioxin have been identified from the plot log V_R versus number of chlorine atoms in the dioxin molecule, which yields a straight line.

GC-MS analysis of PCDDs

The fly ash extract was evaporated to 0.5 ml. and purivied on a silica column (10 cm long, 0,8 cm i.d., containing 2.5 g silica, 70-230 mesh, Merck) by elution with 10 ml of n-hexane. The hexane eluate was concentrated to 0,5 ml by evaporation under a nitrogen stream at about 50°C and transferred on an alumina column (5g alumina, basic, Woelm) using 10ml portions of 2% and 50% methylene chloride in n-hexane. The former eluate contains mainly PCBs, whereas the latter, containing PCDDs and PCDFs, was carefully concentrated to 1 ml and used for GC-MS analysis.

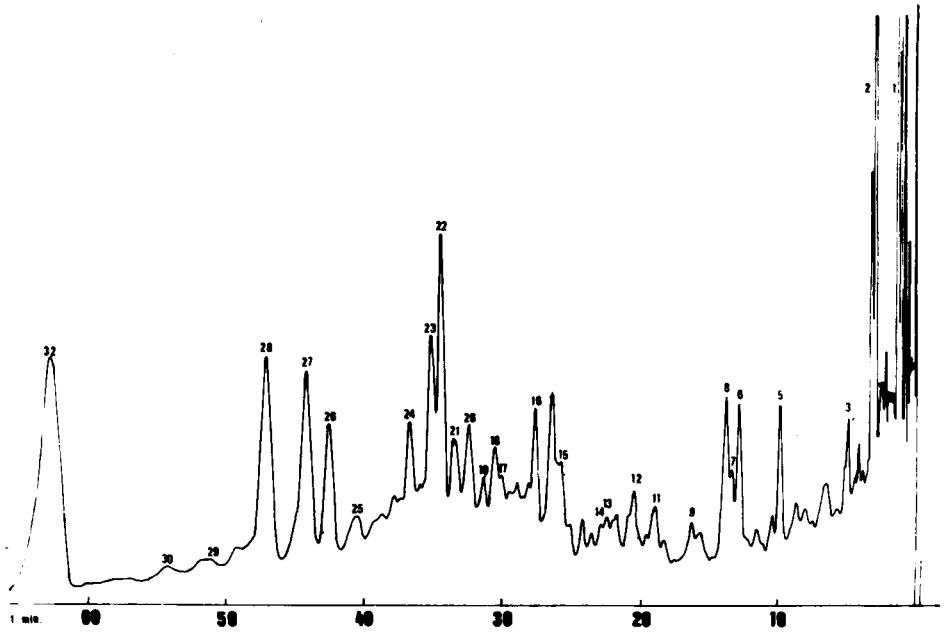

Fig. 2. Gas chromatogram of a benzene extract of fly-ash collected at the electrostatic filter. 2,4,5,8,9,10,11,12,14 and 15 PCB; 13 Tetra-CDD, 17 Penta-CDD; 21,23,24 Hexa-CDD, 27,28 Hepta-CDD, 32 Octa-CDD; Other peaks not identified.

The effectiveness of the outlined procedure for separating PCDDs from PCBs and other compounds was checked by G.C. analysis of the sample before and after the treatment (Fig. 2 and 3); only the peaks due to PCDD were detected.

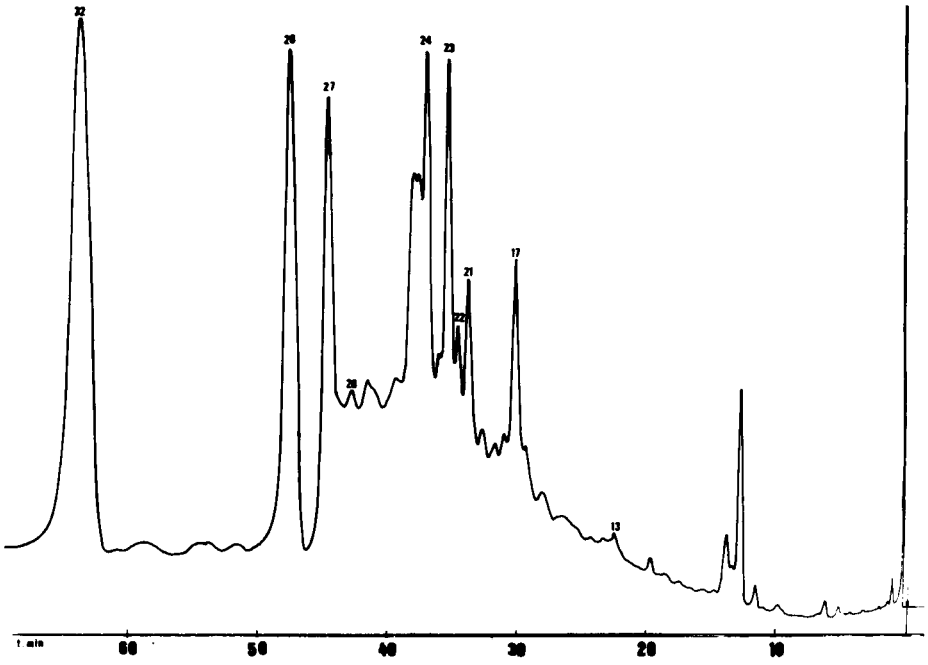

Fig. 3. Gas chromatogram of the same sample of fig. 2 after clean up and elution on alumina-column.

Recovery experiments with known amounts of synthetic standards, added to fly ash, gave yields of about 70-80%. The purified extract was analyzed on the same column used for direct GC-analysis. The column was coupled to a LKB-MS mass spectrometer with an electron-impact ion-source (50 eV) at 240°C. Helium was used as carrier gas (3 kg/cm^2). Benzene samples were injected by keeping the column temperature at 240°C.

The PCDDs identification was carried out by monitoring molecular ions at m/e 320, 322, 324 (tetra-); 356, 358, 360 (penta-); 388; 390, 392 (hexa-); 424, 426, 428 (hepta-), and 456, 458, 460(octa-CDD).

RESULTS AND DISCUSSION

Gas chromatograms of the benzene extract of fly-ash collected in the electrostatic filter (Fig. 2) are very similar to the ones obtained with the ash (Fig.4) and the particulated matter in the fumes (Fig.5): PCDDs are the most abundant compounds whereas PCBs are in smaller concentration. Tetra-, penta-, hexa-, hepta-, and

octa-CDD are always present, the higher chlorinated dioxins being in larger amount.

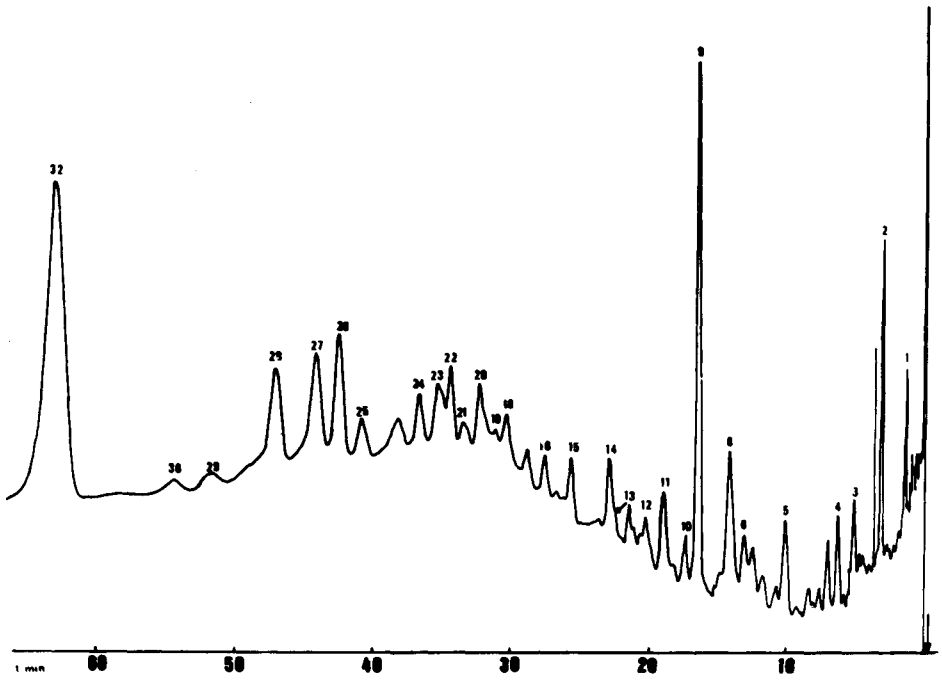

Fig. 4. Gas chromatogram of a benzene extract of ash.

The gas chromatograms relative to the water condensate and to the toluene trap show the presence of a variety of chlorinated compounds which have not been identified but no PCDD.

An attempt has been made to develope a quantitative approach for estimating the content of the incinerator emissions in terms of chlorinated compounds. However, as not all of the compounds have been identified (some of the corresponding standards were not available) it has been necessary to introduce some approximations. The data collected for PCBs were obtained by comparing the total area of the peaks attributed to the chlorobiphenyls present in the samples to the total area of a standard solution of Arochlor 1248. This Arochlor was chosen because it appears to exhibit the closest G.C. pattern.

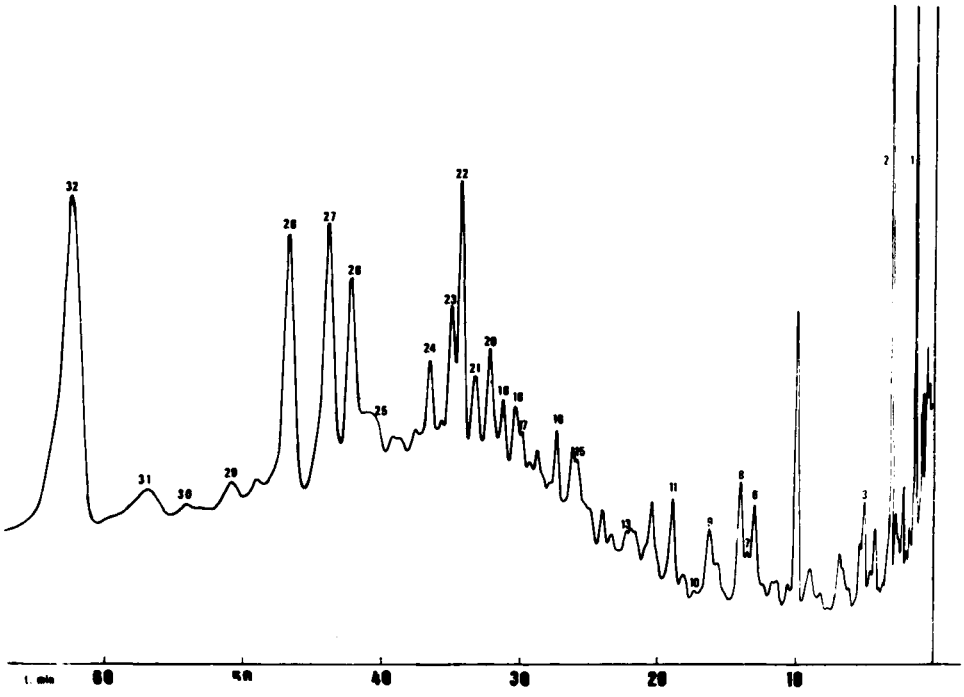

Fig. 5. Gas chromatogram of a benzene extract of particulated matter in the fumes

The PCDDs content was determined using the relative response factors calculated by plotting the area of the peaks given by tetra-, hexa-, and octa-CDD standard solutions versus chlorine atom number. The unidentified peaks in the elution range of PCDDs have been assumed to have the same gas chromatographic response.

Table I gives the data relative to the chloro-organic compounds found in various emissions. The highly chlorinated compounds are present in large concentration and octa-CDD is by far the most abundant.

In order to obtain information on the reactions occurring in an incinerator and in order to establish if most of the compounds detected in the emissions were already present in the wastes, a large sample of the same garbage, which was going to be incinerated, was taken, crushed, homogenized and dried at 60°C. A benzene extract after clean-up on a silica-column yielded the gas chromatogram shown in fig. 6. Peaks before number 4 are due to chlorophenols and isomeric species whereas the others are due to pesticides and PCB's. No other compounds have been observed after 40 minutes. This indicates that PCDDs were not originally present and their formation should be attributed to pyrolitic reactions of the precursors.

TABLE I Concentration of chloro-organic compounds in the emissions of an urban incinerator

Peak No.	Compounds	Dry wastes (ng/g)	Ash (ng/g)	Fly ash (ng/g)	Fume part. matter (ng/g)
	PCB_{TOT}	200	550	1.100	7.800
13	2,3,7,8- and/or 1,2,3,4-tetra-CDD	-	trac.	trac.	40
16	not id.	-	2	9	115
17	penta-CDD	-	4	6	110
20	not id.	-	4	7	160
21	1,2,4,6,7,9-hexa-CDD	-	2	7	150
22	not id.	-	6	24	400
23	hexa-CDD	-	4	15	250
24	1,2,3,6,7,8-hexa-CDD	-	4	7	140
26	not id.	-	15	210	-
27	hepta-CDD	-	8	25	400
28	hepta-CDD	-	8	30	500
32	octa-CDD	-	35	50	900

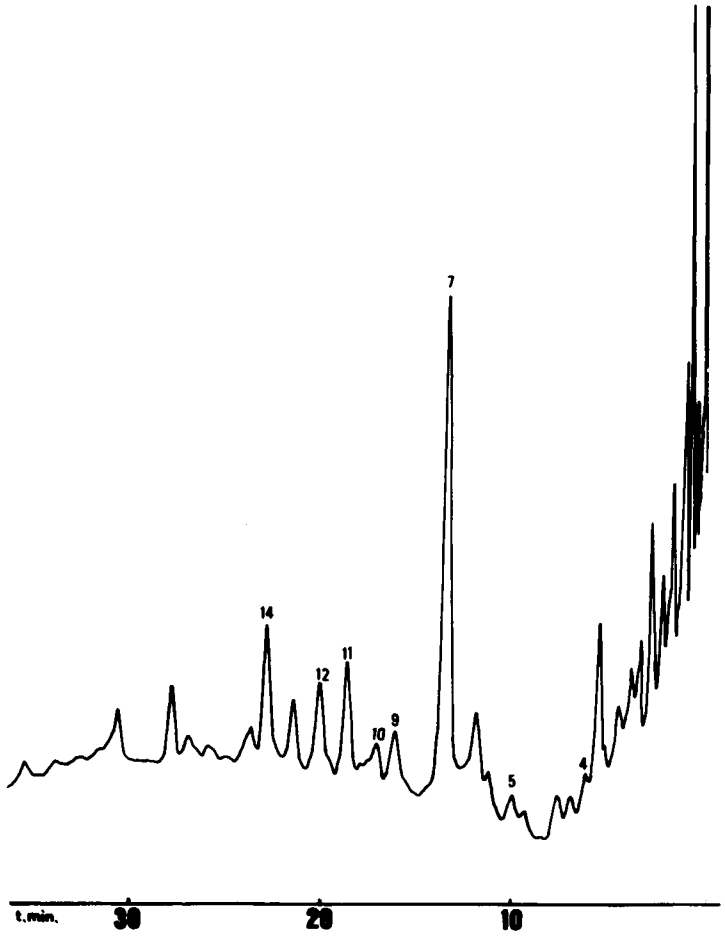

Fig. 6. Gas chromatogram of the benzene extract of a garbage sample.

CONCLUSIONS

The data reported in this work refer to the determination of organochlorine compounds in the emissions of an incinerator of a large city, which is burning mainly household and some industrial refuse but no chemical waste as a role. Also the heterogeneity of the garbage thus treated could result in appreciable variations and the behaviour of other incinerators would have to be investigated in order to draw conclusions of a general validity. Nevertheless, the figures obtained might be taken as indicative of a typical urban incinerator. Its emissions in terms of organochlorine compounds are strongly affected by the nature of the input material and namely by its content of precursors as well as by operating conditions, abatement systems and flue gas temperature.

In the emissions, considerable amounts of PCBs and PCDDs are always found. These compounds are in larger concentrations in the particulated matter sampled in the fumes and in the fly-ash. A variety of PCDDs are always present; the ones which are present to a greater extent are the more chlorinated, octachlorodibenzodioxin being usually the most abundant. Since no PCDDs have been detected in the material to be incinerated and these compounds are found in all emissions, definitely reactions occur in the incineration process which yield to the formation of these compounds.

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Polychlorinated Biphenyls and Related Halogenated Compounds

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ABSTRACT

A group of industrial compounds obtained by chlorination of biphenyl and commonly referred to as PCB have become the classical organic pollutants during the last ten years. Their physico-chemical properties and lack of chemical and biochemical reactivity has resulted in the worldwide distribution of these persistent and bioaccumulating compounds. PCB show a range of long-term biological effects and, because of the complexity of the commercial material, are difficult to analyse for accurately. PCB were produced and released into the environment in large quantities and although manufacture and use are now curtailed in most industrialised countries, concentrations of PCB in the environment are still relatively high and analysis for these compounds in air, water, sediment and biota will have to be carried out for some time. The presence of PCB in the environment can also be considered an unplanned, large scale "experiment" which may be used as case study for the behaviour and fate of persistent organic compounds in the environment. For such a global *in situ* study in environmental chemistry, analysis in various regions of the world and in different environmental compartments will yield information on the distribution and disappearance of PCB.
Special attention must be given to potentially toxic impurities in PCB preparations and analytical methods have to be developed for the study of polar metabolites and environmental transformation products of PCB.
Finally, those products which are designed to replace PCB in critical applications deserve our interest. They must be studied before any large scale use may create new problems for the environment.

INTRODUCTION

Among the many chemicals which cause environmental problems, e.g. heavy metals, polycyclic aromatic hydrocarbons (PAH), oil, nitrosamines, fungal toxins and halogenated aromatic and aliphatic hydrocarbons, the group of chemicals containing halogen atoms forms and important and diverse class. It may be sufficient to mention some of these: 1. The stable chlorofluorocarbons (freons) which are suspected of interfering with the ozone layer of the upper atmosphere; 2. The haloforms and related compounds which are formed in the process of water treatment with chlorine; 3. Chlorinated short-chain aliphatic solvents are used in considerable quantities; 4. The large class of chlorinated pesticides, for instance DDT, the cyclodienes, lindane, toxaphene, mirex,

kepone; 5. Most organic flame retardants contain halogen, examples are polybrominated biphenyls (PBB) and the organophosphorous compound Tris which contains six bromine atoms per molecule; 6. The extremely toxic compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds including chlorodibenzofurans; 7. Pentachlorophenol and other chlorinated phenols; 8. Chlorinated benzenes and olefins and last but not least 9. PCB.

There is as yet really no good scientific answer why halogenated and particularly chlorinated compounds appear so prominent on the list of pollutant compounds. Chlorine- and fluorine-substitution appear to make organic compounds persistent, and yet, if one considers the strength of the C-Cl bond which for an aromatic compound is about 86 kcal/mole and in aliphatic compounds somewhat lower (81-84 kcal/mole) there seems to be no simple and obvious chemical reason why these compounds are so stable in the environment since bond energies in molecules containing only carbon, hydrogen and oxygen are in the same order of magnitude, or higher.

The incorporation of chlorine in a molecule makes the compound more lipophilic *i.e.* it aids the partition of the compound from water into the fatty phase of an organism, a physical characteristic important in bioaccumulation; but again, chlorine is not unique in this respect, simple alkyl groups *e.g.* propyl, have the same effect; in other words propylbenzene has approximately the same lipophilicity as chlorobenzene.

The presence of chlorine in a molecule does not necessarily or uniquely increase the toxicity of a compound. Admittedly, one of the most toxic compounds known to man, TCDD, is a chlorinated derivative of a parent compound with low toxicity. But even here other groups can be substituted for chlorine and still give very toxic compounds. Examples are electron withdrawing groups such as the cyano or nitro group.

On the other hand, the carcinogenic properties of benzo(a)pyrene disappear on chlorine substitution, so there is no straightforward correlation between chlorine substitution and toxicity either.

These examples are given to indicate that it is impossible to give simple answers in this area and that fundamental work in environmental and toxicological chemistry is necessary.

Very often organochlorine compounds and organohalogen compounds generally are rather thoughtlessly considered as one group. Even chlorinated compounds are far from being a homogeneous group.

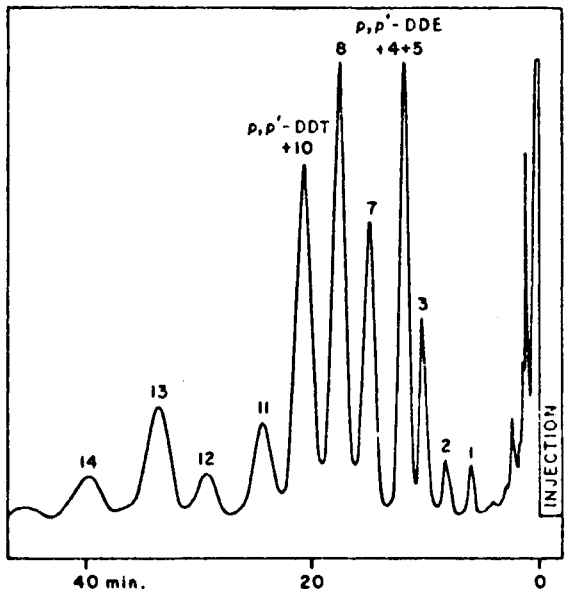
In the following scheme an attempt is made to subgroup organochlorine compounds. The grouping is mainly based on structural characteristics and physical properties. These two features are largely responsible for the fate of these compounds in the environment, and this grouping usually also combines compounds for which similar analytical procedures are applicable.

Classification of some organochlorine compounds.

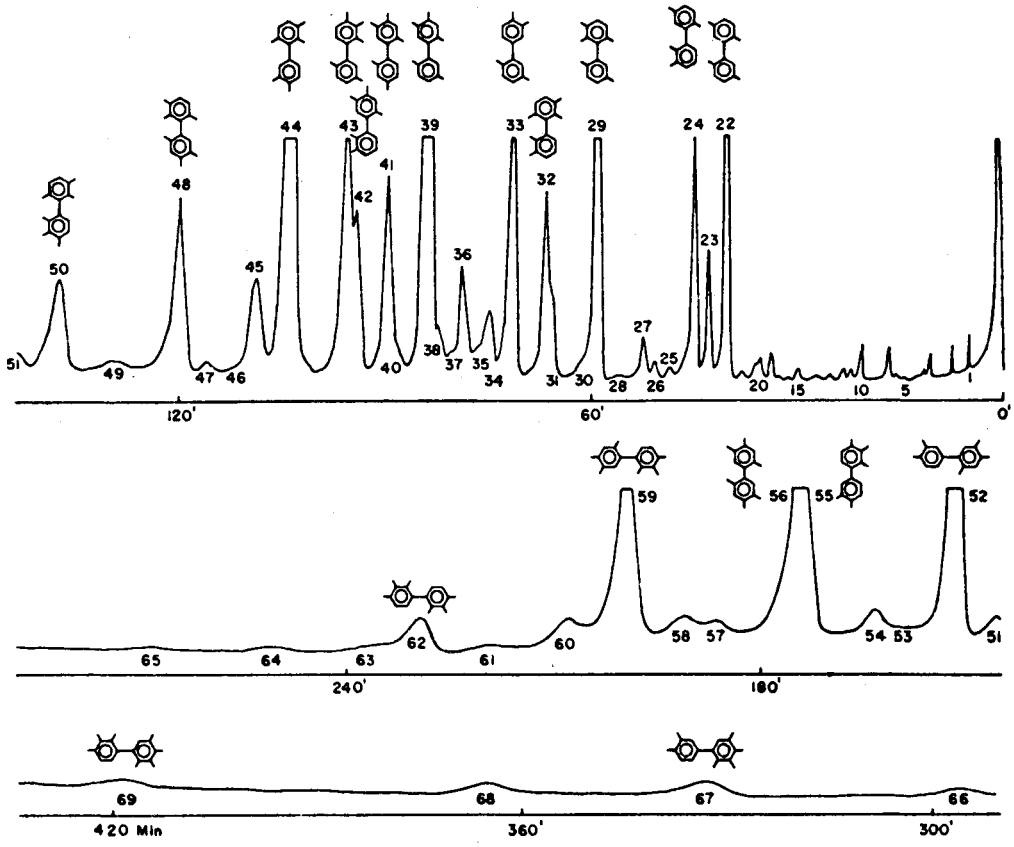
class	examples
chloroaromatic compounds	PCB, PCT, DDT, chlorobenzenes
cycloaliphatic compounds	cyclodiene insecticides, lindane, toxaphene, mirex
saturated chloroaliphatic compounds	chloro derivatives of lower alkanes (CCl_4, methylchloroform)
unsaturated chloroaliphatic compounds	vinylchloride, trichloroethylene, hexachlorobutadiene
fluorocarbons	chlorofluoromethane propellants and refrigerants

This paper deals mainly with polychlorinated biphenyls. However, we do not intend to review the analytical methodology which is available today but would rather like to use the PCB to illustrate the complexity of an environmental problem and to indicate where analytical chemistry can contribute to the understanding of such a problem.

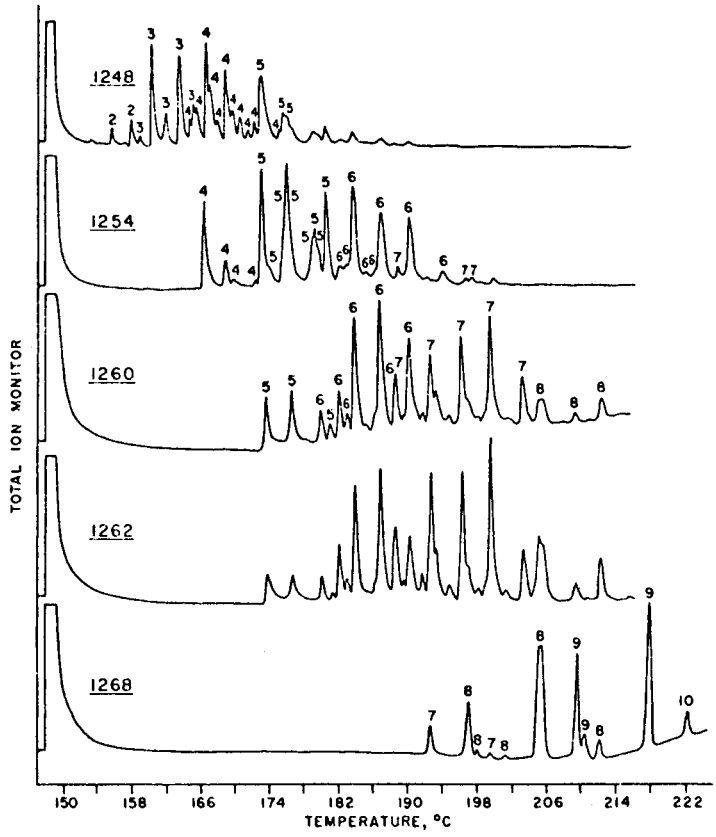
In the following figure, a historic gas chromatogram related to the discovery of PCB in environmental samples is shown and the following chromatograms illustrate the development of GC-technology since that time in a chronological order.



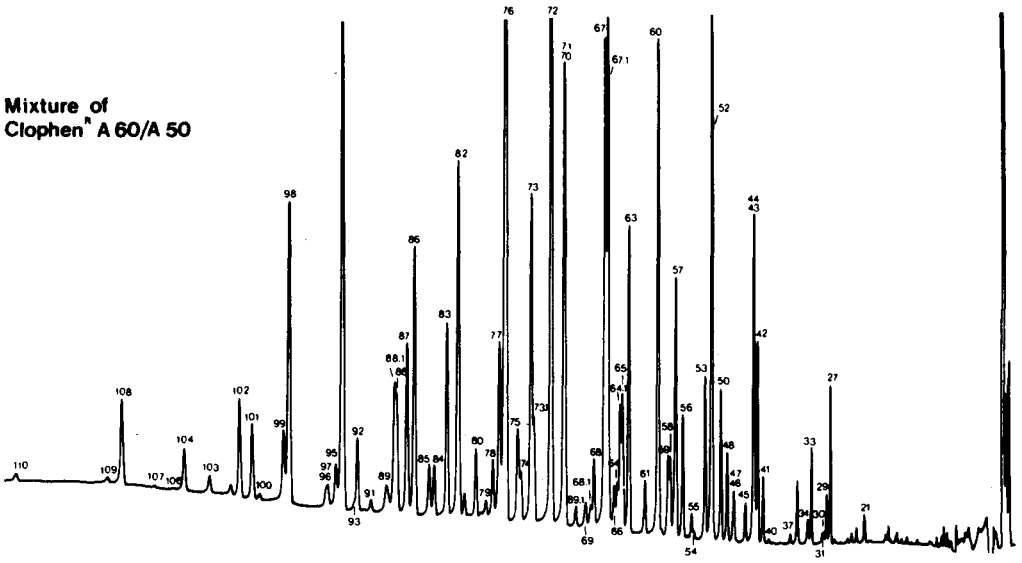
1966: Gas chromatogram from purified extract from white--tailed eagle. All peaks but DDT and DDE were unknown (courtesy of Dr. Jensen).



1971: Capillary column chromatogram of Aroclor 1254. (courtesy of Drs. Sissons and Welti)



1972: Capillary column chromatograms of some Aroclors. Numbers above the peaks designate the number of chlorine atoms per biphenyl molecule (courtesy of Dr. Stalling).



1978: Glass capillary gas chromatogram with an electron capture detector (ECD) of a mixture of Clophen A60/A50 (1:2.2) (courtesy of Prof. Ballschmiter).

ANALYSIS AND THE ENVIRONMENTAL EXPOSURE CONCENTRATION.

The hazard of a particular compound (e.g. PCB) to a given living organism depends on the compound's intrinsic toxicity and the concentration (availability, exposure concentration) of the compound in the environmental subcompartment leading to exposure.

A compound may enter the environment, then be transported and transformed to other structures by biological or photochemical action. The compound or its transformation product(s), depending on the distribution and fate in air, water, soil or sediment may become available for different organisms in various environmental subcompartments at different concentrations depending on its "environmental behaviour". Very volatile compounds, for instance, will not be available to fish in high concentrations and the same is true for compounds which are biodegraded rapidly. Compounds which absorb strongly to sediment or have very low solubility in water, again, will not be found in high concentrations in water, but may become available at low concentrations, over prolonged periods of time from the "reservoir" sediment.

The environmental exposure concentration depends strongly on thermodynamically controlled distribution equilibria and rate constants for the various distribution processes. It is perhaps needless to point out in this respect how important a role analytical chemistry plays here.

In the following section an attempt is made to present an overall environmental fate profile (*cf. 1*) for PCB, featuring data and information important and necessary for the understanding and prediction of concentrations of PCB in various environmental subcompartments and the occurrence of important metabolites and related transformation products.

Such an environmental fate profile enables the presentation of available data in a concise and logical fashion and readily points out important gaps in our knowledge to be filled by analytical and related research.

In this section only factors leading to exposure, including the fate within an exposed organism (metabolism, bioaccumulation) are considered, whereas toxic effects are not.

ENVIRONMENTAL FATE PROFILE FOR PCB.

The following entries are used:

1. General information. Information of interest about PCB which does not fit any other section of the profile.
2. Production, use, impurities, release into the environment.
3. Physical constants. Important for predicting environmental behaviour.
4. Physical processes - transport, adsorption.
5. Transformations in the environment; photochemical reactions.
6. Transformations in the environment - biodegradation.
7. Overall environmental fate (sinks, residence time, environmental half-lives).
8. Concentration in the environment.
9. Uptake, bioaccumulation, excretion (clearance).
10. Metabolism.

Because of the large number of relevant references, use is made only of books, reviews and reports (1-16) except in some special cases. Most items in the following sections are presented in an abbreviated style and may serve as a quick reference system.

1. General Information.

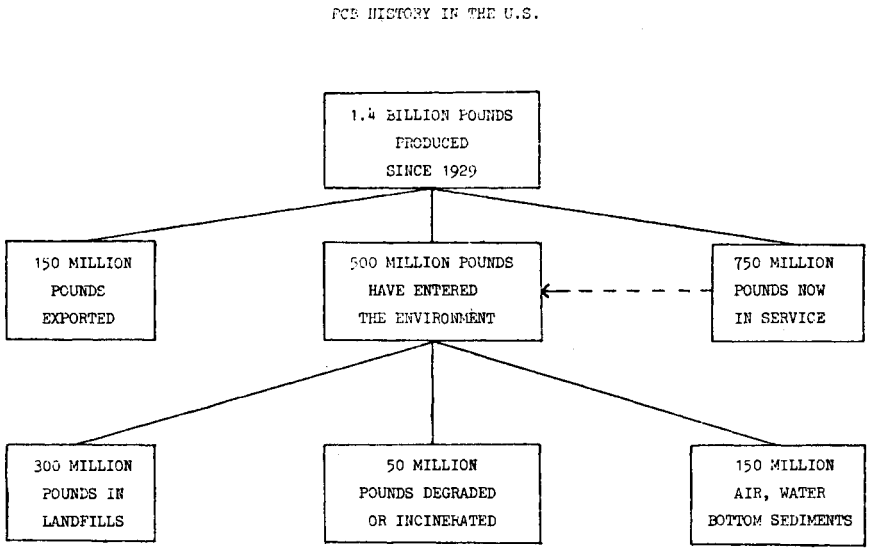
PCB has been produced and used since 1930; it was recognized as an environmental contaminant only in 1966 (applications of advanced analytical techniques). Despite curtailment of PCB use, it is still present in the environment and will be present for an undefined period of time.
The unique environmental properties of PCB and related organochlorine compounds become evident when the total amount of PCB production and loss into the environment is compared with similar figures for an organic bulk-contaminant: oil. It has been estimated (17) that for 1970 about 11 x 10^6 tons of the transported crude oil finds its way into sea water; this means 0.5% of the total amount in 1970 (2,2 x 10^9 tons). In the peak year of PCB production (1971), 5 x 10^4 tons were produced world-wide (environmental loss data are not available on a yearly world-wide basis). In spite of the discrepancy of the abovementioned amounts, PCB has become a global pollution problem with world-wide distribution, whereas oil has remained a local (even if large scale) problem. The environmental fate properties of PCB (lack of efficient degradation mechanisms, dispersion behaviour, lipophilicity) are responsible for this.

2. Production, Use, Release, Impurities.

PCB are synthesized by chlorination of biphenyl. PCB production: US, 1970, 39 x 10^6 kg; 1971, 16 x 10^6 kg; 1972-1974, 18-19 x 10^6 kg; world production: 50 x 10^6 kg in 1971. PCB was used as a heat transfer fluid, plasticizer,

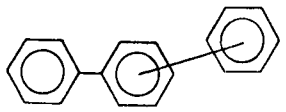
dielectric coating for capacitors, hydraulic lubricant.

Routes of polychlorinated biphenyls into the environment: 1. Spills and losses in the manufacturing of PCB or PCB-containing fluids; 2. Vaporization or leaching from PCB-containing formulations; 3. Leaks from sealed transformers and heat exchangers; 4. Leaks of PCB-containing fluids from hydraulic systems which are only partly sealed; 5. Disposal of waste PCB or PCB-containing fluids.
The present pollution of air and water in the U.S.A. has been caused by an estimated 150 million pounds of past PCB production. Two times as much is deposited in land fills and five times as much is still in service and may eventually be released into the environment, independent of further production of PCB. This is outlined below.

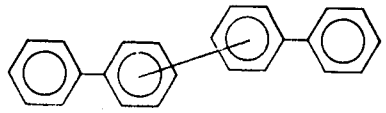


Impurities: Chlorodibenzofurans and chloronaphthalenes were shown to be impurities in most commercial PCB preparations some time ago. Recently (18), other possibly toxicologically important impurities were discovered in used fluids containing PCB.

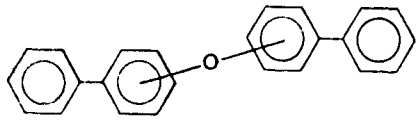
IMPURITIES IN USED PCB MIXTURES



Cl_5 - Cl_8 TERPHENYLS



Cl_4 - Cl_9 QUATERPHENYLS



Cl_7 - Cl_{10} QUATERPHENYL ETHERS

3. Physical Constants Important for Predicting Environmental Behaviour.

Solubility in water: Individual chlorobiphenyls from ~ 5000 ppb (2-chlorobiphenyl) to 0.016 ppb (decachlorobiphenyl).
Vapour pressure: 4,4'-dichlorobiphenyl 1.12 x 10^{-3} mmHg (30°C), 1.34 x 10^{-3} mmHg. The vapour pressure of PCB mixtures (Aroclor 1242 to 1268) ranges from 4 x 10^{-4} to 10^{-6} mmHg (25°C).

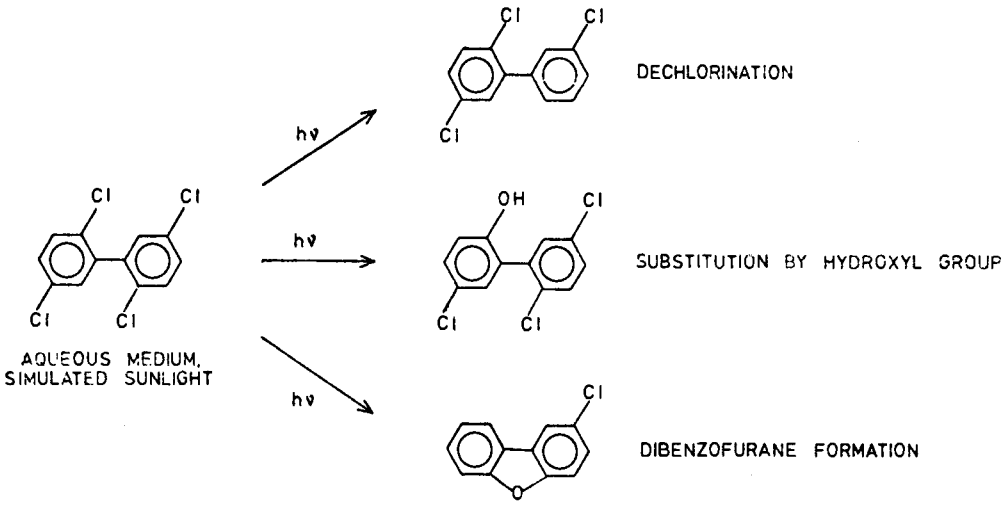
Partition coefficient: (log P n-octanol/water) 4,4'-dichlorobiphenyl 5.5; 2,2',4,5,5'-pentachlorobiphenyl 6.1; 2,2',4,4',5,5'-hexachlorobiphenyl 6.7. (25°C).

4. Physical Processes - transport, adsorption.

Adsorption: The distribution coefficient of PCB between marine sediments and seawater is reported as 2 x 10^5.
Transport: PCB has a different moving pattern trough marine ecosystems than DDT. Three atmospheric removal processes result in ocean deposition: rain, dry fall-out and vapour phase deposition onto surfaces. 1.3 - 1.8 x 10^6 kg of PCB are discharged to the atmosphere each year from North America by vaporization and burning (7-9% of the peak U.S. production). Fluxes of aerosol PCB (1254) ranging from 2 x 10^{-9} to 9 x 10^{-7} g/m^2.day in La Jolla, California (1972). Most dry deposition of aerosol PCB introduced into the troposphere occurs within 100 km from the source. The rate of vertical mixing in the ocean is too slow to account for the quantities of PCB found in Atlantic & Mediterranean abyssal sediments if one assumes only transport in dissolved state; it may be carried to deep ocean sediments by rapidly sinking particles. 1.2 x 10^{-4} g/m^2.yr of PCB accumulates in sediments of the Santa Barbara Basin. Sedimentation rate in the open ocean is considerably lower.

5. Transformations in the Environment: chemical and photochemical reactions.

Photodegradation/oxidation: Upon irradiation with UV radiation, the PCB's degraded fastest in hexane, then water and reaction was slowest in benzene. The process is concentration independent; products are most likely formed by dechlorination. Photolysis of 2,4,6,2',4',6'-hexachlorobiphenyl with λ > 310 nm in hexane readily degraded the starting compound and gave products formed by loss of chlorine, rearrangement and condensation. Relative photolabilities of some chlorobiphenyl isomers: (24 hrs irradiation with λ > 310 nm in hexane), remaining of 3,4,3',4'-tetrachlorobiphenyl 32%, of 2,6,2',6'-tetrachlorobiphenyl 29%, of 2,5,2',5'-tetrachlorobiphenyl 33%, of 2,4,5,2',4',5'-hexachlorobiphenyl 3.8%, of 2,3,4,5,2',3',4',5'-octachlorobiphenyl less than 1%; products are formed by reductive dechlorination and some polymeric material is also present. Photolysis of chlorobiphenyls in water gives hydroxylated (chloro)biphenyls along with dechlorination compounds. Photoreactivity is greater with higher chlorine content of the PCB's; indoor and outdoor results were identical. When ortho chlorine substituents are present, small amounts of chlorinated dibenzofurans are formed also.
The most important photoreactions of chlorobiphenyls are depicted for a tetrachloroderivative below.



PHOTOCHEMICAL REACTIONS OF PCB

6. Transformations in the Environment: biodegradation.
^{14}C-labelled 2,2',5-tri-, 2,2',5,5'-tetra- and 2,2',4,5,5'-pentachlorobiphenyl were studied in a laboratory model ecosystem for degradation pathways and biomagnification in alga, snail, mosquito and fish: the trichlorobiphenyl was degraded in all the organisms much more rapidly than the tetra- and pentachlorobiphenyl; Pentachlorobiphenyl was considered to be as persistent as DDE. Aroclor 1254 and pure isomers (hexachlorobiphenyl) were degraded by Pseudomonas sp. The degradation was dependent on the length of incubation, purity and degree of chlorination of the biphenyl molecule. PCB stimulated growth and oxygen uptake. Biodegradation is assumed to be not important; however, significant microbial degradation of PCB has been reported by several investigators. Recently, degradable and non-degradable chlorobiphenyl structure-types have been distinguished by comparing PCB samples from the environment with the standard preparations (19).

Recalcitrant PCB-substitution patterns. The most abundant in 54% Cl- and 60% Cl-PCB mixtures are underlined

4-
2,4-; 3,4-; 3,5-;
2,3,4-; 2,3,5-; 2,4,6-; 3,4,5-;
2,3,4,5-; 2,3,4,6-; 2,3,5,6-;
2,3,4,5,6-

Degradable PCB-substitution patterns. The most abundant in 54% Cl- and 60% Cl-PCB mixtures are underlined

2-; 3-;
2,3-; 2,5-; 2,6-;
2,3,6-;

7. Overall Environmental Fate. (sinks, residence time, environmental half-lives)

Once chlorobiphenyls are in the atmosphere, they are adsorbed on particulate matter and possibly photolyzed. Total loss of PCB in the atmosphere ca. 1.5 - 2.5 x 10^5 tons/yr. Final sink for PCB is predicted to be degradation in the atmosphere with some fraction being buried in the underlying sediments (of e.g. Lake Michigan).
Residence time: Air: half-life in the troposphere of aerosol highboiling chlorinated hydrocarbons released near the surface ca. 5 hrs; water: half-life of PCB: 1242: 5.96 hr; 1248, 58.3'; 1254, 1,2' and 1260, 28.8' for evaporation from water phase.

8. Concentrations in the Environment and Biota.

An overview of PCB concentration ranges in the environment and food are given in the following tables:

PCB LEVELS IN THE ENVIRONMENT

	p.p.m.	p.p.b.	p.p.t.
MARINE AIR			0.05 - 2.0
CONTINENTAL AIR			0.002 - 14.0
OCEAN WATER (sub surface)			0.03 - 2.5
OCEAN WATER (surface)		0.005 - 4.2	
RAINFALL			10 - 100
OCEAN BOTTOM SEDIMENTS		2 - 800	
SEWAGE SLUDGE	1 - 100		
MUD DEPOSIT BELOW PCB-FACTORY DRAINAGE	380 - 82,600 (!)		

PCB LEVELS IN SOME ANIMAL SPECIES AND MAN (in p.p.m.)

ATLANTIC COD	0.001 - 0.016
PINK SALMON	0.011 - 0.028
TUNA	0.023 - 0.032
SEAL (blubber)	1.08 - 2.46
POLAR BEAR (fat)	2.1 - 7.8
MAN (abdominal fat)	n.d. - 18
(milk)	0.2 - 3.0
(adipose tissue)	0.2 - 5.1

MAXIMUM LEVELS OF PCB IN FOOD IN THE U.S.A. (in p.p.m. of extractable lipids)

	1973	1974	1975
FISH	123	16.8	9.0
MILK	1.6	2.3	1.9
EGGS	n.d.	11.0	n.d.
CHEESE	0.5	2.8	n.d.
PROCESSED FOODS	19.2	n.d.	n.d.
ANIMAL FEEDS	199.5	n.d.	n.d.

9. Uptake, Accumulation, Elimination (clearance).

Uptake + accumulation: Uptake and bioconcentration are a function of two important factors: 1. Lipophilicity (lipid/water partition coefficient) and 2. Resistance to degradation by enzymatic processes. PCB are accumulated to a high degree by marine organisms. Marine mammals and birds accumulate PCB to levels determined by their position in the food web, and the highest levels occur in specialised predator species.

Benthic organisms can accumulate PCB through ingestion of sedimentary particles or by absorption from direct contact with sediments and/or the surrounding waters.
After rapid uptake of 2,2,5'- and 2,4',5-trichlorobiphenyl by goldfish (Carassius auratus) during the first few days, a slower accumulation takes place, with an estimated 60 days for reaching an equilibrium (with a constant concentration of trichlorobenzenes in water (20).
Fish probably the main sources of PCB's for mother's milk (Sweden).

Bioconcentration factors: For 2,2',5-trichlorobiphenyl 21,000 and for 2,4',5-trichlorobiphenyl 30,000 in goldfish (23 days)(20).

Biomagnification coefficients for some marine organisms: Pinfish (Lagodon rhomboides): 22,000 (14-28 days); sheepshead minnow (Cyprinodon variegatus): 16,333 (4 weeks) oysters, 101,000 - 165,000; shrimp: 26,000;Grass Shrimp: 11,000 (7 days), 8,065 (2 weeks); pink shrimp: 2,500 (4 days).

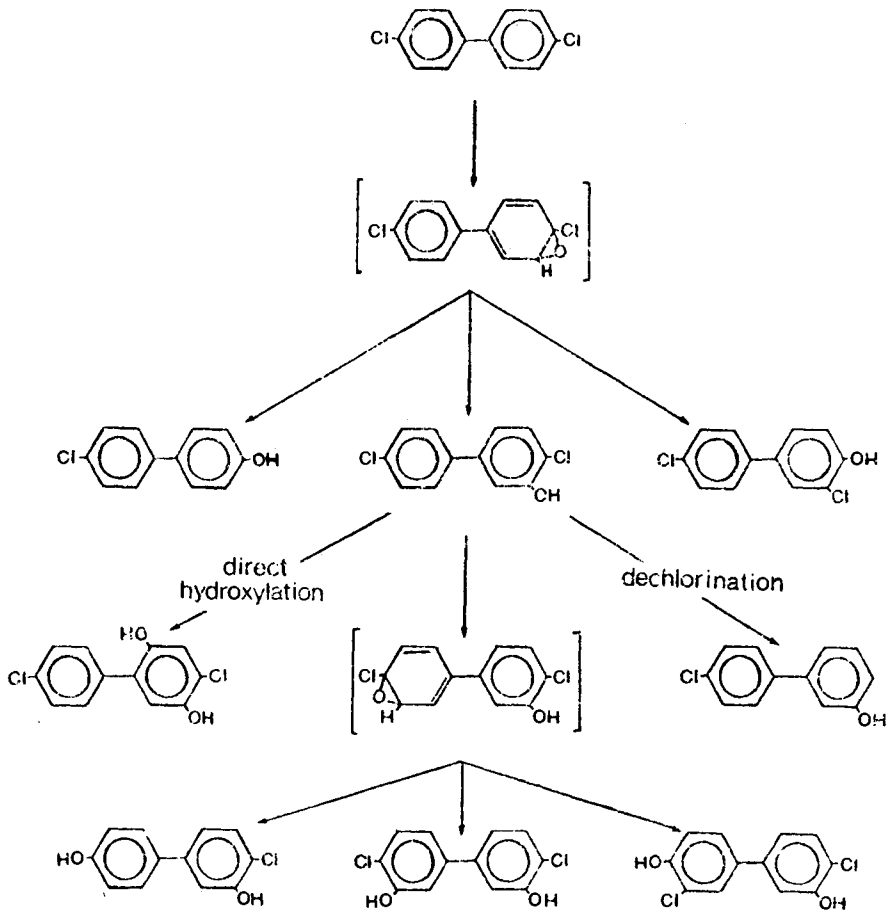
Clearance of 2,2',5- and 2,4',5-trichlorobiphenyls from goldfish has a half-life of 13 and 28 days, respectively (20).

10. Metabolism.

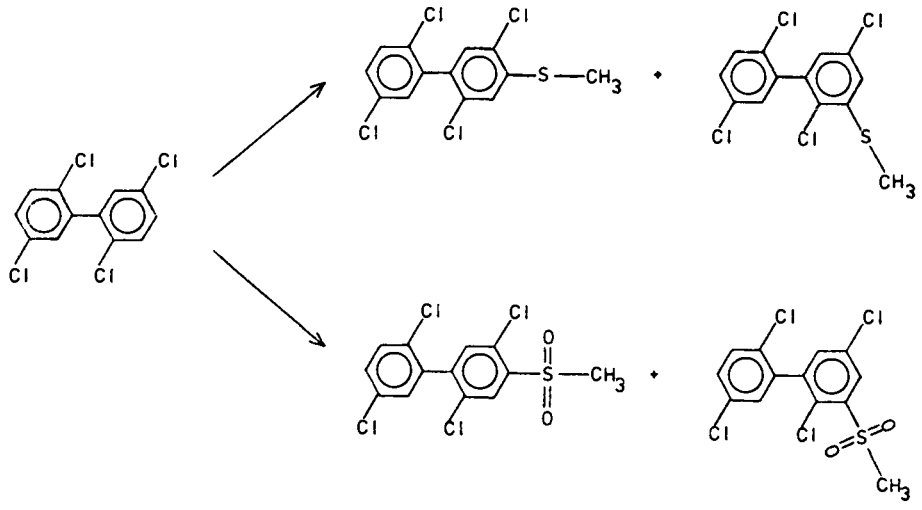
PCB are metabolized by a variety of mammalian organisms to hydroxylated derivatives including conjugates. In most instances arene oxide mechanisms are involved and metabolism of PCB with two unsubstituted ortho positions is thus

facilitated. Thio-derivatives and sulfoxides have been found in metabolism studies and natural samples.

Examples of metabolism to hydroxylated derivatives and sulfur containing compounds are shown below (21,22).



Rat metabolism of 4,4'-dichlorobiphenyl.



SULFUR CONTAINING METABOLITES OF 2,2',5,5'-TETRACHLOROBIPHENYL IN THE RAT

COMPOUNDS TO REPLACE PCB.

From the data given below it is evident that as of July 1979 PCB will no longer be a commercial commodity in the U.S.A. In some countries this ban is already in force (e.g. Sweden) and eventually PCB will not be allowed in all industrialezed countries at least.

CUTOFF DATES FOR CERTAIN PCB USAGES AS ESTABLISHED BY TOSCA (U.S.A.)

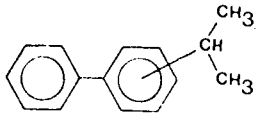
JANUARY, 1978 ALL MANUFACTURING, PROCESSING, DISTRIBUTION IN COMMERCE, AND USE MUST BE IN A TOTALLY ENCLOSED MANNER.

JANUARY, 1979 NO FURTHER MANUFACTURING OR IMPORTING OF PCB IS ALLOWED.

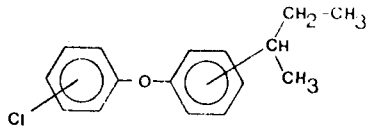
JULY, 1979 NO FURTHER PROCESSING OR DISTRIBUTION IN COMMERCE OF PCB IS ALLOWED.

A number of compounds and mixtures are suggested and already used as replacements for PCB in transformers, capacitors and as hydroaulic- and heat transfer fluids.

Although most of these compounds have been tested for possible environmental and health effects, analytical procedures for their determination are generally not readily available. Some of these compounds are shown below.



I



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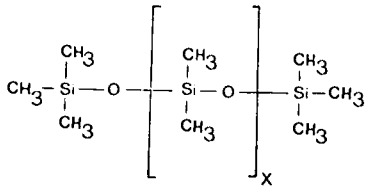

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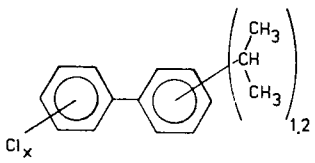

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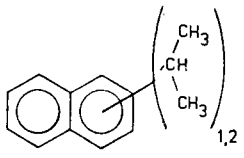

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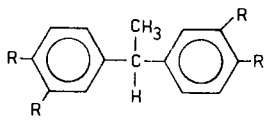
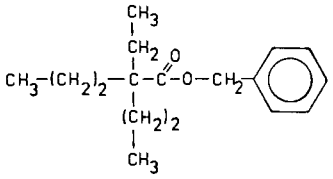
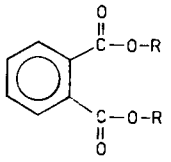


R = Cl, CH_3

VI



VII



R = octyl, isononyl

VIII

Additional information regarding these PCB-replacement compounds are presented in the following table:

Compound	Chemical or industrial name	Use pattern
I	Isopropylbiphenyl	in carbon-less copying paper, capacitors and as heat transfer fluid
II	Monochloro sec-butyldiphenyl ether	-
III	Dimethyl siloxanes	in transformer fluids
IV	Chloralkylenes	-
V	Di-isopropylnaphthalenes	in carbon-less copying paper
VI	Iralec (R = Cl) DXE (R = Me)	-
VII	Benzyl Neocaproate	small scale use
VIII	Alkylphthalates R = octyl, isononyl	electrical applications

In addition, there are some classes of compounds used as PCB replacement compounds; these are mixtures of chlorinated paraffins, refined paraffinic mineral oils and synthetic hydrocarbons. The composition of these mixtures has usually not been determined.

ACKNOWLEDGEMENT: We gratefully acknowledge valuable discussions with Drs. M.Th.M. Tulp.

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Characterization of Chlorinated Hydrocarbons in the Marine Environment: Sampling and Analytical Aspects

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ABSTRACT

The methodology adopted to obtain marine samples for chlorinated hydrocarbon residue analysis in estuarine and coastal zones is presented. Sampling devices include a large volume filter and specifically designed plankton nets to avoid surface film contamination. A modified gas chromatographic spectral analysis technique to determine individual component concentrations for polychlorinated biphenyls is discussed, together with an automated computational scheme for data reduction and presentation.

KEYWORDS

PCBs, chlorinated hydrocarbons, marine sampling, water sampler, chlorobiphenyls, quantitation-PCB, plankton sampler, GC spectral analysis, automated quantitation procedure, PCB sampling.

DESCRIPTION OF SAMPLING EQUIPMENT

Throughout our work in assessing the distribution and bioaccumulation of chlorinated hydrocarbons in the marine environment we have used specially designed sampling gear to avoid contamination and insure sample integrity at the low concentrations encountered. A brief description of this equipment is described below together with a computational scheme for determining polychlorinated biphenyl residues in natural samples.

Water sampling devices. For deep water sampling (>5m) whole water and suspended particulate samples were collected with 53-liter stainless steel samplers (Young, Buddemeir, and Fairhall, 1969) modified by the replacement of the drain valve with a 1/2 inch stainless steel Swage-Lok^{R} quick disconnect. For shallow water sampling (<5m) a mechanical 10 liter stainless steel sampler suited for gas overpressure filtration was used. Schematic diagrams of the latter device are shown in Figure 1.

Large volume filter (LVF). A filtering system compatible with the deep water sampler described above was designed to collect large quantities of suspended particulate matter with minimum contamination potential (Pavlou and co-workers, 1978). This system attained filtering rates in excess of 200 liters/hour in most marine areas sampled. A schematic diagram of the LVF is shown in Figure 2. The intake line consists of 1.25 cm seamless aluminum tubing connected with Teflon-lined neoprene tubing. Larger organisms and/or foreign material are excluded by a 40 mesh screen suction strainer at the end of the intake line. The LVF can also be connected directly to the discharge part of the deep water sampler, thus allowing discrete sampling, more depth flexibility and the ability to measure directly any other desired water quality parameters from the same water sample. A diagram of the stainless steel filter chamber is shown in Figure 3. The filters are 20x25 cm Reeve Angel 934 AH, contain no organic binders and have defined retention characteristics with a median retention of approximately 0.5 um. The filters were always precleaned by combusting at 500°C for at least 24 hours prior to use.

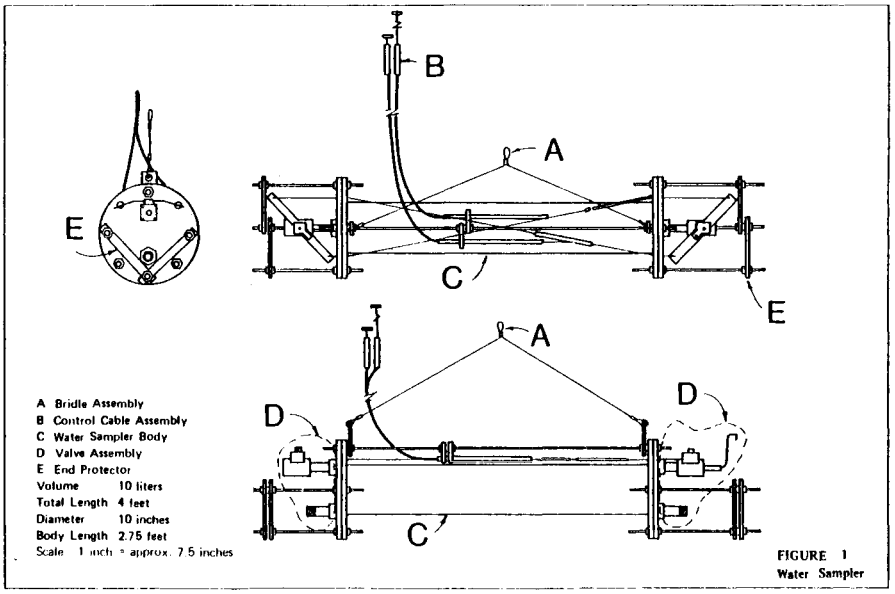

FIGURE 1
Water Sampler

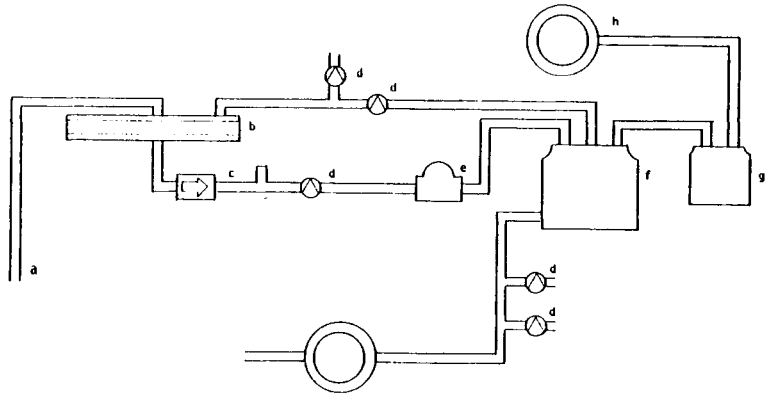

FIGURE 2 Schematic Diagram of the Large Volume Filter System: a, intake line; b, filter chamber; c, check valve; d, shut-off valve; e, totalizing water meter; f, main ballast tank; g, water trap; h, vacuum pump; i, water pump.

Plankton Nets. Due to potential contamination of plankton samples from trace compounds accumulating in surface films, special nets for zooplankton and phytoplankton were constructed to avoid such interferences (Clayton and Pavlou, 1978). A schematic diagram of the zooplankton device is shown in Figure 4.

To evaluate the capability of the protected closed net for excluding surface film contaminants, samples were collected with both the modified net and an open unprotected net. Although the average chloribiphenyl concentration was higher in the open net samples, the differences were not statistically significant (Table 1).

In most of the marine areas where we used this net, contamination by chlorinated hydrocarbons residing on the surface film did not account for greater than 1% increase in the residue levels measured in zooplankton. The contamination potential in other regions where high pollutant loads on surface films has been observed might be quite significant. The quantities of various trace organic compounds which might be expected to enter an open net under conditions observed by other investigators are summarized in Table 2. In view of the above considerations, the design of plankton collection devices which minimize surface contamination is important for obtaining meaningful data.

SPECTRAL ANALYSIS TECHNIQUE FOR POLYCHLORINATED BIPHENYLS

A modified gas chromatographic spectral analysis technique has been developed for determining individual chlorobiphenyl component responses (Dexter and Pavlou, 1976).

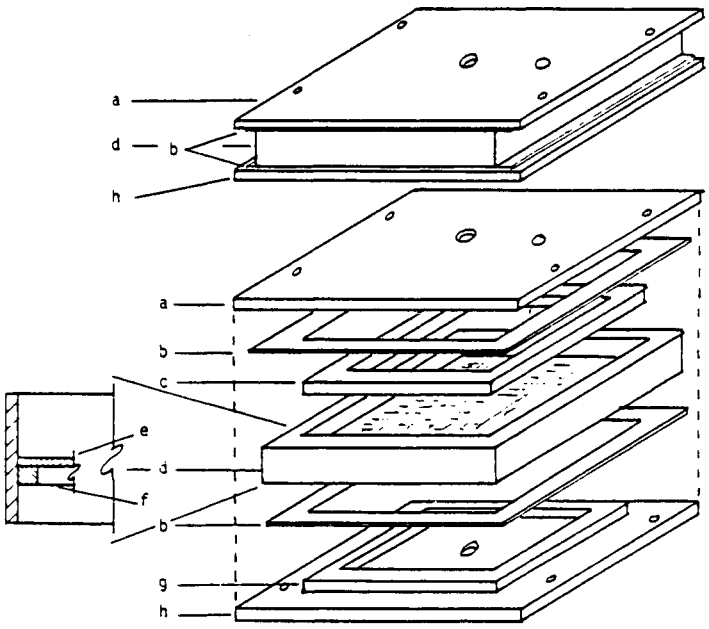

FIGURE 3 A Diagram of the Large Volume Filter Chamber: a, top plate; b, silicone rubber gaskets; c, filter retaining ring; d, filter support section; e, screen; f, screen support flange; g, bottom plate covering flange; h, bottom plate.

The response, in peak area, A'_i, of any particular chlorobiphenyl, i, in a flame ionization detector can be expressed as

$$A'_i = R'_i n_i \quad (1)$$

where R'_i is a response factor and n_i is the number of moles of i injected.

Since

$$n_i = m_i/M_i \quad (2)$$

where m_i is the mass injected and M_i is the molecular weight of i,

$$A'_i = R'_i m_i/M_i \quad (3)$$

or

$$m_i = A'_i M_i/R'_i \quad (4)$$

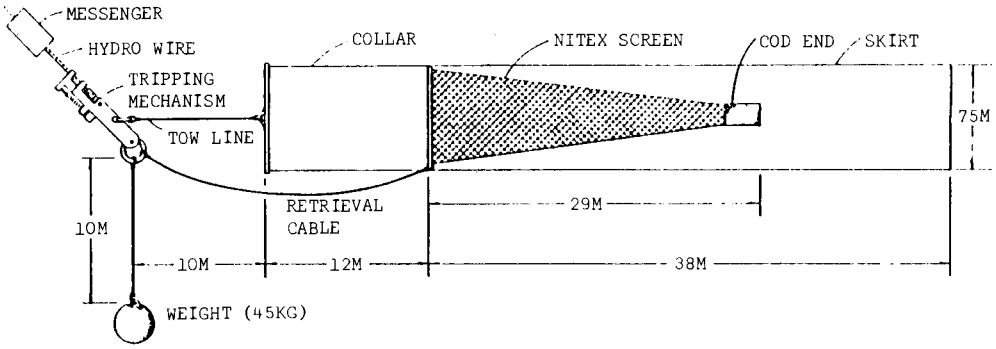

FIGURE 4a
A Schematic Diagram of the Protected Closed Net, Deployed Stage

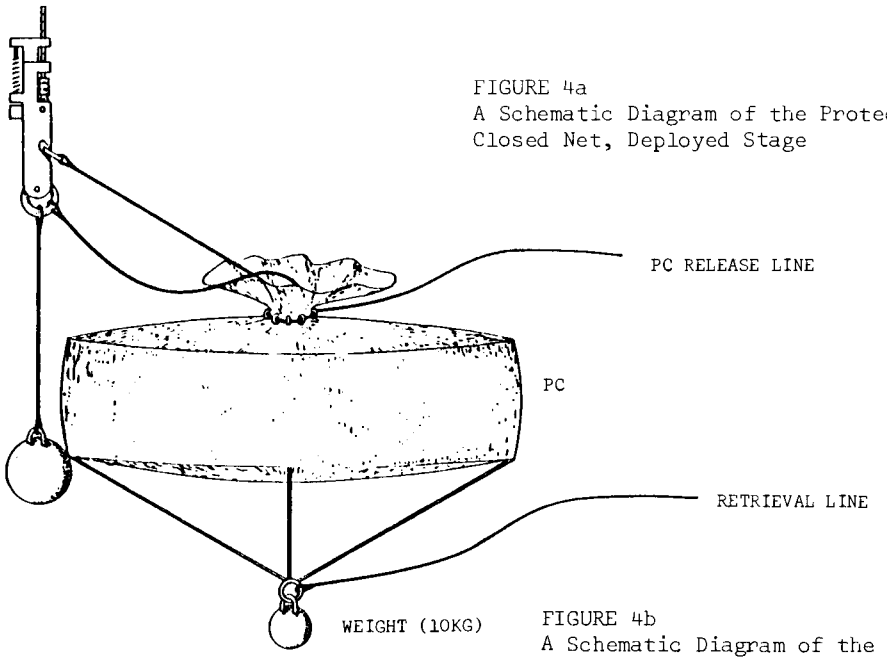

FIGURE 4b
A Schematic Diagram of the Protected Closed Net, Launching Stage

Further, by definition, the total mass injected m_t is equal to the sum of the masses of all components:

$$m_t = \Sigma m_i = \Sigma A_i' M_i / R_i \tag{5}$$

and the mass fraction, F_i, of component i is then

$$F_i = \frac{m_i}{m_t} = \frac{A_i' M_i / R_i'}{\Sigma A_i' M_i / R_i'} \tag{6}$$

TABLE 1

Chlorinated hydrocarbon concentrations* in zooplankton samples† from Elliott Bay, Puget Sound.

Protected Closed Net			Open Net		
Sample No.	PCB‡	ΣDDT§	Sample No.	PCB	ΣDDT
T-1	14.7	0.6	T-4	15.2	0.6
T-2	9.6	0.5	T-5	12.1	0.5
T-3	7.8	0.4	T-6	14.0	0.6
$\bar{x} \pm s$	10.7 ± 3.6	0.5 ± 0.1		13.8 ± 1.6	0.6 ± 0.1

*Concentrations are expressed in 10^{-9} g/g wet weight; $\bar{x}$ and s denote the mean and standard deviation, respectively.

†In all samples hydromedusae and copepods were the predominant zooplankton forms with small numbers of amphipods, crab zoea larvae, chaetognaths, and juvenile euphausiids.

‡PCB was quantitated as Aroclor(R) 1254; a commercial chlorobiphenyl mixture with an average chlorine number of approximately 5.0.

§Abbreviations: p,p'-DDT, 1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl) ethane; p,p'-DDE, 1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethylene; p,p'-DDD, 1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethane; ΣDDT = p,p'-DDT + p,p'-DDE + p,p'-DDD.

TABLE 2.

Estimated quantities of surface trace organics entering the open plankton net.

Trace organic	Quantity (g) entering net	Location	Investigator
Chlorinated Hydrocarbons (PCB)*	5.5×10^{-7}	Narragansett Bay, R.I. (heavy slick)	Duce and Co-workers, 1972
	7.0×10^{-9}	Elliott Bay, Puget Sound	Clayton, Pavlou, and Breitner, 1977
Petroleum tar lumps	0.86×10^{-3}	Northwest Atlantic	Morris (1971)
	17.2×10^{-3}	Mediterranean Sea	Horn, Teal, and Backus, 1970
Plastic particles	0.25×10^{-3}	Sargasso Sea	Carpenter and Smith (1972)

*These values were computed assuming the upper 150 μ of the water column was sampled in surface film studies (Garrett 1965).

For the flame ionization detector, however, it has been observed that the molar response factors are nearby the same for all PCB components, i.e.,

$$R_1' = R_2' = \ldots = R_i' \tag{7}$$

therefore

$$F_i = \frac{m_i}{m_t} = \frac{A_i' M_i}{\Sigma_i A_i' M_i} \tag{8}$$

Applying this analysis to a standard chlorobiphenyl mixture, one can generate a series of F_i values corresponding to the individual analytical components of the standard. Thus, for any known mass of standard m_t, and the appropriate F_i, the specific mass of component i be determined from

$$m_i = m_t F_i \tag{9}$$

If the same standard is chromatographed under identical conditions, but with EC detection, similar component separation will be obtained but with different response characteristics.

The corresponding EC response factor, R_i, is then simply expressed as

$$R_i = \frac{A_i}{m_i} = \frac{A_i}{m_t F_i} \tag{10}$$

where A_i refers to the area in the EC trace. Once an R_i value has been determined from the analyses of the standard, its corresponding m_i in an unknown sample can be easily obtained from relation (10).

where A_i refers to the area in the EC trace. Once an R_i value has been determined from the analyses of the standard, its corresponding m_i in an unknown sample can be easily obtained from relation (10).

Although F_i is only internally consistent and independent of the absolute FID response, R_i is governed by the operational parameters which effect A_i. Therefore, the calibration utility of R_i is limited since simultaneous injections of standards would be required with each unknown. This shortcoming can be overcome by calculating the response of each component relative to an operationally convenient external standard. This relative response is defined as the sensitivity ratio

$$S_i = \frac{R_i}{R_{st}} \tag{11}$$

where R_{st} refers to the EC response of the external standard.

Combining relations (10) and (11), the injected mass of each component in a sample extract, u, can be determined as follows.

$$m_{i(u)} = \frac{A_{i(u)}}{S_i R_{st}} \tag{12}$$

$A_{i(u)}$ is now the EC peak area of the ith component in the sample and R_{st} is the response for the sequentially injected external standard. The corresponding concentration in the sample can be easily computed as

$$[i\text{-CB}]_u^x = \frac{m_{i(u)}}{LU_x} \tag{13}$$

where L is the volume fraction of the sample extract injected in the chromotograph and U_x is the quantity of sample extracted.

Since the isomeric identities of each of the PCB components are largely unknown and difficult to determine, final data reduction is presently most reasonable in terms of the concentrations of CB of the same degree of chlorination, [N-CB]. This is obtained simply as the sum of [i-CB] for all i of the same N.

For comparison of the CB content between samples, the mass fraction, F_n, of the difference N-CB can be calculated as

$$F_n = \frac{[N\text{-CB}]}{[t\text{-CB}]} \tag{14}$$

where [t-CB] is the sum of concentrations of all CB residues.

It can be seen from the above considerations that from measured areas and the appropriate sensitivity ratios, one can determine directly the N-CB abundance in any environmental sample; plots of F_n versus N can then be constructed to provide a direct representation of the CB distribution within any region sampled.

Table 3 shows the mole fractions, X_i, the molar concentrations, C_i, the mass fractions, F_i, and the degree of chlorination, N, for the components of Aroclor 1242, 1254, and 1260, respectively. From F_i, M_i, and N, the %N-CB composition, the average molecular weights and the chlorine mass percent of each standard were

TABLE 3

Summary of Quantitation Variables for Chlorobiphenyl Standards

		X_i			C_i			F_i			F_n		
i	N	1242	1254	1260	1242	1254	1260	1242	1254	1260	1242	1254	1260
1	0	0.001			0.0053			0.0004			0.0004		
2	1	0.009			0.0476			0.0060					
3	1	0.004			0.0212			0.0026			0.0095		
4	2	0.044			0.2329			0.0378					
5	2	0.011			0.0582			0.0093					
6	2	0.110	0.005		0.5822	0.0232		0.0937	0.0034		0.1408	0.0034	
7	3	0.008			0.0423			0.0079					
8-9	3	0.158	0.005		0.8363	0.0232		0.1552	0.0039				
10	3	0.061			0.3229			0.0607					
11-12	3	0.168	0.004		0.8892	0.0186		0.1652	0.0033				
13-14	3	0.098	0.002		0.5186	0.0093		0.0965	0.0017		0.4855	0.0089	
15-16	4	0.097	0.086	0.003	0.5134	0.3995	0.0128	0.1077	0.0828	0.0024			
17-18	4	0.104	0.035	0.001	0.5505	0.1591	0.0043	0.1159	0.0344	0.0061			
19-20	4	0.023	0.059		0.1217	0.2741		0.0259	0.0560				
21	4	0.071	0.004[1]	0.036[2]	0.3758	0.3902[1]	0.1538[2]	0.0786	0.0802[1]	0.0120[2]			
22-24	4	0.033	0.170[1]	0.050[2]	0.1747	0.7897[1]	0.2136[2]	0.0365	0.1622[1]	0.0442[2]	0.3642	0.1732	0.0031
25-27	5		0.086	0.007		0.3995	0.0299		0.0814	0.0063			
28	5		0.098	0.039[2]		0.4553	0.1666[2]		0.0935	0.0376[2]			
29-31	5		0.141	0.163		0.6550	0.6963		0.1342	0.1595		0.6190	0.0762
32-33	6		0.053	0.122		0.2462	0.5212		0.0620	0.1188			
34	5		0.063	0.082[2]		0.2927	0.3503[2]		0.0675	0.0797[2]			
35	6		0.027	0.120		0.1577	0.5126		0.0911	0.1170		0.1531	0.5126
36	7		0.013	0.082		0.0604	0.3503		0.0173	0.0827			
37-40	7		0.019	0.112		0.0872	0.4784		0.0249	0.1198			
41-42	7			0.102			0.4357			0.1007			
43-44	7			0.080			0.3417			0.0856		0.0422	0.4018

[1] These components are pentachlorobiphenyls (N=5)

[2] These components are hexachlorobiphenyls (N=6)

calculated and compared with the manufacturer's specifications and literature values in Table 4. The good agreement indicates that the F_i values and component N designations are accurate.

Comparisons of the mass percent of each N-CB determined from these studies with values from the literature fail to show complete agreement. However, this is probably indicative of variations in the CB content of commercial PCB mixtures. Therefore, F_i values are only directly applicable to the particular standard analyzed.

It should be noted that individual GC peaks, while relatively invariant in retention time, represent CB of different N value in different standards. Since the degree of chlorination for each component cannot always be determined in environmental samples, S_i is useful only if a single value can be assigned to each spectral component. Figure 5 shows a plot of S_i as a function of relative retention time, $t_{r(i)}$; both quantities are normalized to p,p'-DDE. S_i initially increases rapidly with increasing $t_{r(i)}$ and approaches a maximum at longer retention times. It was encouraging to note that components of the same retention time, even though of different N value, gave similar S_i values. S_i values were therefore averaged for those components which appeared in more than one standard. In order to facilitate computation of S_i in terms of $t_{r(i)}$ an empirical equation

$$S_i = 0.614 \log t_{r(i)} + 0.783 \qquad (15)$$

was generated by regression analysis. The correlation coefficient was 0.927. Since the scatter in the data was mainly due to the difficulty in accurately measuring components of small peak area, it was felt that the S_i values calculated from the equation could give better results.

From the quantitation of known standard injections via S_i analysis, it became apparent that much of the variation in S_i for components of both low and high N values was real. Use of a combination of the averaged values at the spectral extremes and the S_i values calculated from the empirical equation for the intermediate points gave the best agreement between the known and S_i-calculated [t-CB] and [N-CB] values for a series of injected standards. The values of S_i thus obtained are compiled in Table 5.

From these S_i values, a series of single and mixed Aroclor standards were injected and quantitated. The results are shown in Table 6. Since the instrument precision, determined from peak height comparisons of repeated injections of a single standard, is approximately $\pm$ 2%, the quantitation by the S_i technique is quite accurate. The net analytical precision for the GC analysis, including instrument response fluctuations, precision in the reading of the volume injected, and planimetry errors, was approximately $\pm$ 5%.

The computational scheme described above has been computerized to facilitate the reduction of the voluminous raw data. Figure 6 shows an overall flow diagram for the whole program. The program is written in FORTRAN IV and requires the availability of a free format input subroutine. It was developed on a CDC 6400 computer system, and is now available at URS for potential users. A sample input and output are shown in Figure 7 and 8.

TABLE 4
Comparison of Mass Percent Composition for Aroclor Standards Among Various Investigators

	Clayton, Pavlou, and Breitner (1977)			Ugawa, M., A. Nakamura, and T. Kashimoto (1973)			Webb and McCall (1973)			Thurston (1971)			Monsanto (1972)		
M-CB	1242	1254	1260	1242	1254	1260	1242	1254	1260	1242	1254	1260	1242	1254	1260
1	0.95						1.1			3			1	0.1	
2	14.08	0.34		7.79[†]	0.35[††]	0.12[†††]	16.95			13			16	0.5	
3	48.55	0.89		59.66	2.76	1.52	39.19			28			49	1.	
4	36.42	17.32	0.31	28.11	8.93	1.63	31.83	13.8		30	11		26	21	
5	0.1	61.90	7.62	1.42	60.28	5.30	8.71	61.9	11.53	22	49	12	8	40	
6		15.31	51.26		22.07	35.69		23.3	46.14	4	34	38	1	23	
7		4.22	40.18		5.62	44.70		1.0	34.84		6	41		6	
8			0.1			10.14			6.10			0	100		
9						0.03									
Σ-CB	100.05	99.98	100.00	96.98	100.00	100.00	97.78	100.00	98.61	100.0	100.0	100	100.0	99.5	
Σ Cl	42.1	54.97	59.76	42.9	54.60	60.70	43.4	54.7	60.11	46.1	55.7	60.1	43.1	54.1	60
M	263.0	325.17	366.6	256.1	330.09	376.1	260.6	327.9	365.1	270.8	336	374.3	264.0	326.0	

[†]Penechlor 300 (Kanegafuchi Chemical Industrial Co. Ltd., Japan)

[††]Penechlor 500

[†††]Penechlor 600

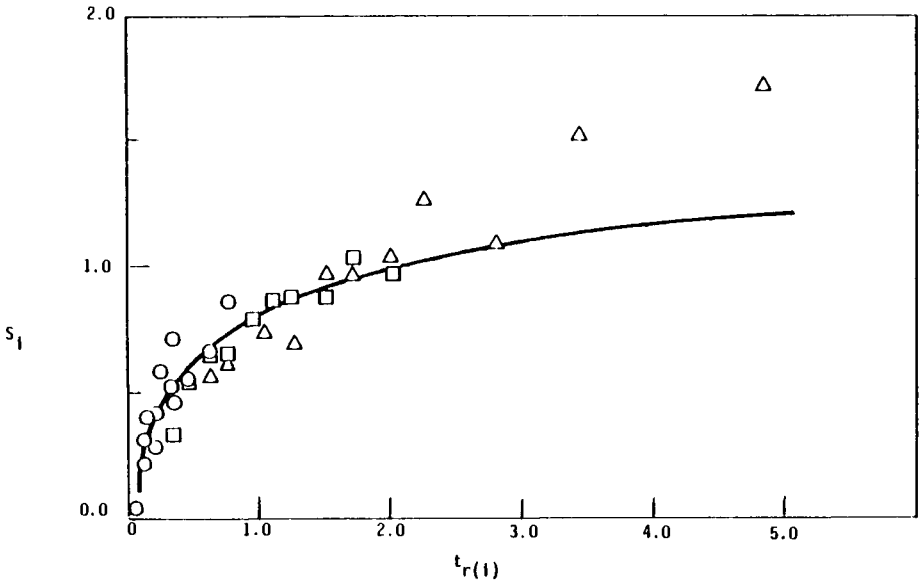

FIGURE 5 Plot of the Sensitivity Ratio, S_i, Versus the Relative Retention Times, $t_{r(i)}$, of the Components of Aroclors 1242, 1254, 1260. Both S_i and $t_{r(i)}$ were Normalized to p,p'-DDE.

Table 5 Values of the Relative Retention Time, $t_{r(i)}$, and Sensitivity Ratio, S_i, for Aroclor Standard Components

i	$t_{r(i)}$	S_i
1	0.003	0.000
2	0.005	0.000
3	0.074	0.000
4-6	0.115	0.170
7-9	0.199	0.300
10-14	0.299	0.506
15-16	0.397	0.537
17-18	0.498	0.597
19-21	0.636	0.662
22-24	0.794	0.722
25-27	0.970	0.775
28-30	1.114	0.812
31	1.291	0.851
32-33	1.508	0.893
34	1.729	0.929
35	2.013	0.970
36	2.297	1.263
37-40	2.824	1.094
41-42	3.471	1.504
43-44	4.833	1.708

Table 6 Results of the Quantitation of CB Residues by EC-GC Using S_i Technique

Standard	$m_{t(inj)} \times 10^{-10}$ g	$\Sigma m_i \times 10^{-10}$ g	% Δ
1242	2.061	2.058	- 0.15
1254	2.674	2.713	1.46
1260	2.279	2.221	- 2.64
1242 + 1254	3.254	3.389	4.15
1242 + 1260	3.170	3.255	2.68
1254 + 1260	2.891	2.943	1.80
1242 + 1248 + 1260	4.986	4.910	- 1.52

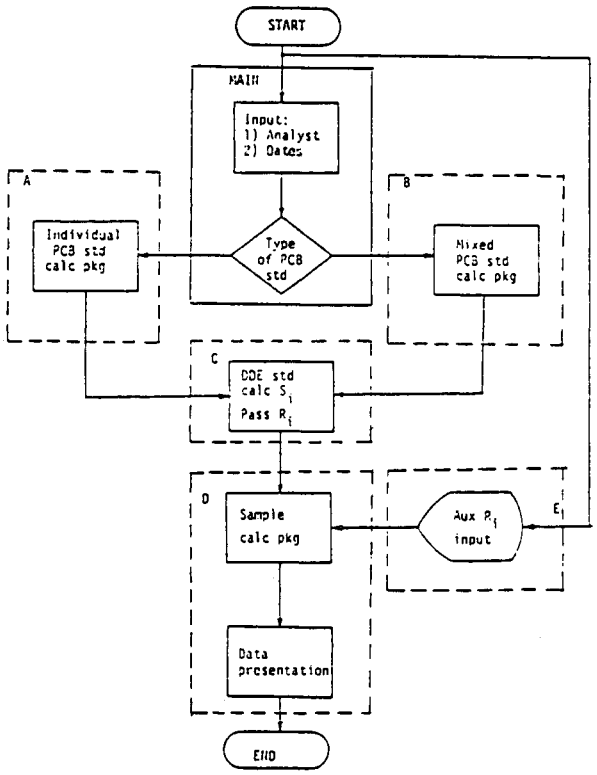

FIGURE 6 Overall Flow Scheme for Data Reduction.

```
ANY (MORE) CORRECTION ? ENTER YES OR NO: NO

ENTER: TYPE, NO INJ, NORM FAC, FIN VOL(ML):
?2 3 10.33 2.03

DATA ACCEPTED: TYPE NO INJ     NORM FAC     FIN VOL
                    2       3      1.0330E+01   2.0300E+00

ANY (MORE) CORRECTIONS? ENTER YES OR NO: NO
ENTER THE CORRESPONDING INJ VOL(UL) FOR EACH INJ:
?2.06 2.2 2.11

DO YOU WANT TO OUTPUT SAM INJ     DATA TABLE?
ENTER YES OR NO: NO
ENTER AREAS FOR EACH CLUSTER:
ENTER ONLY INTEGERS FOR AREAS, MIN=1,MAX=999999 !!!
ENTER INJ NO  1,CLUSTER NO  1: 838 3524
ENTER INJ NO  1,CLUSTER NO  2: 9785
ENTER INJ NO  1,CLUSTER NO  3: 45056
ENTER INJ NO  1,CLUSTER NO  4: 39852
ENTER INJ NO  1,CLUSTER NO  5: 30665
ENTER INJ NO  1,CLUSTER NO  6: 67398
ENTER INJ NO  1,CLUSTER NO  7: 63507
ENTER INJ NO  1,CLUSTER NO  8: 27315
ENTER INJ NO  1,CLUSTER NO  9: 55537
ENTER INJ NO  1,CLUSTER NO 10: 62137
ENTER INJ NO  1,CLUSTER NO 11: 38648
ENTER INJ NO  1,CLUSTER NO 12: 42475
ENTER INJ NO  1,CLUSTER NO 13: 27450
ENTER INJ NO  1,CLUSTER NO 14: 19709 9556
```

FIGURE 7
Sample of Selected Input Sequence

```
          GROUP CH   DATA TABLE:
RUN DATE: 6 APR 77   CALC DATE: 4 12 77  102  PERSON:RND
SAMPLE LABEL=.PCB-342-16-64-1-2
SAMPLE ID=A3301      SAMPLE TYPE= 2
NORMALIZING FACTOR:1.0300E+01       WITH UNITS DRY GRAM
FINAL VOL(ML):2.0300E+00
CONC IS GM NOR PER DRY GRAM
CL=NUMBER OF CHLORINES
CL AVG MI    STDDEV MI AVG CI    STDDEV CI FRAC C    FRAC M    CHLORINES
 2 8.739E-08 1.271E-10 1.751E-08 2.546E-11 2.745E-02 2.745E-02 2.000E+00
 3 4.320E-07 2.098E-09 8.656E-08 4.204E-10 1.357E-01 1.357E-01 3.000E+00
 4 8.454E-07 2.108E-08 1.694E-07 4.224E-09 2.655E-01 2.655E-01 4.000E+00
 5 1.139E-06 6.030E-08 2.282E-07 1.208E-08 3.577E-01 3.577E-01 5.000E+00
 6 4.313E-07 3.206E-08 8.643E-08 6.424E-09 1.355E-01 1.355E-01 6.000E+00
 7 2.489E-07 8.636E-09 4.988E-08 1.730E-09 7.818E-02 7.818E-02 7.000E+00

TT 3.184E-06 1.165E-07 6.380E-07 2.335E-08 1.000E+00 1.000E+00

AVG GROUP CH WRITTEN ON TEMPORARY FILE SPAREB
```

FIGURE 8
Sample of Output Tables

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Determination of Phenylurea Herbicides and their Metabolites by High Performance Liquid Chromatography

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ABSTRACT

A method for the determination of a herbicide and its metabolites is described. Furthermore it is shown that reversed-phase high-performance liquid chromatography is a powerful method for the separation und determination of several herbicides in a single run.

KEYWORDS

Herbicide residues determination, phenylurea herbicides, high-performance liquid chromatography

INTRODUCTION

Substituted phenylureas are well known and widely used as selective herbicides. Therefore it is necessary to be able to determine residues of phenylurea herbicides in soil, water, and plant material.
Gas-liquid chromatography as well as colorimetry have been well established for the determination of herbicide residues (1). But the disadvantage of colorimetry lies in its lack of specifity as well as in a simultaneous analysis of many herbicidal compounds. When using gas-liquid chromatography one has to take account of thermal decomposition products especially when dealing with phenylurea herbicides. This gap can be filled by high-performance liquid chromatography. A lot of workers have already used liquid chromatography for the determination of phenylurea herbicides (2-5).
This work wants to show that it is possible either to determine a herbicide and its metabolites or a simultaneous determination of several herbicides.

EXPERIMENTAL

Apparatus. A Hewlett-Packard liquid chromatograph 1084 A with a built-in UV-detecor (254 nm) was used.

Columns. Stainless-steel columns (250 mm x 4.6 mm i.d.) were carefully cleaned and then filled with reversed-phase material by the balanced density method. The material used was
a) LiChrosorb RP 18 5 µm (E.Merck)
b) self-prepared material by reaction of octadecyltrichlorosilane with LiChrosorb SI 100 10 µm (E.Merck)
c) self-prepared material by reaction of octadecyltrimethoxysilane with LiChrosorb SI 100 10 µm for which the authors thank Dr. Hemetsberger.

Solvents. All solvents used were of analytical reagent grade.

Chemicals. The herbicides Fenuron, Methoxuron, Monuron, Carbaryl, Monolinuron, Methoxuron, Monuron, Carbaryl, Monolinuron, Metobromuron, Diuron and Linuron were commercially available by Fluka.
Methabenzthiazuron and its metabolites were a gift by the Bayer AG for which the authors thank Dr. Jarczyk.
Al herbicides were dissolved in methanol.

RESULTS AND DISCUSSION

Our aim is the determination of methabenzthiazuron (MBT) and its metabolites. Methabenzthiazuron is the active ingredient of Tribunal® commercially available by the Bayer AG.
In plants MBT is degraded to N-hydroxymethal-N'-methyl-N'(2-benzothiazolyl)-urea (I) as well as to N'-methyl-N'-(2-benzothiazolyl)-urea(II) (6)

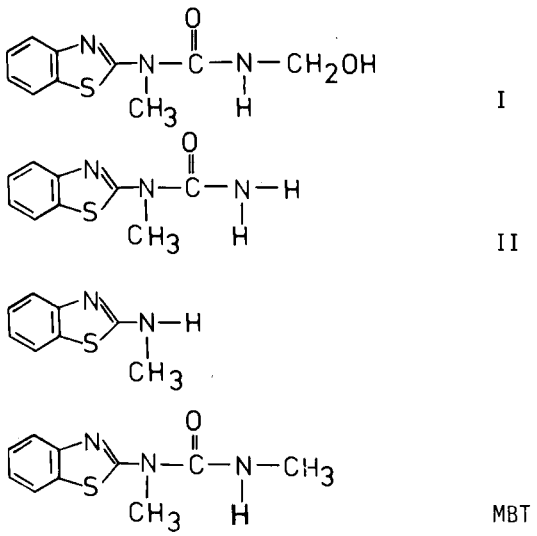

Fig. 1 Formula of methabenzthiazuron and its metabolites.

Figure 2 shows the separation of these substances. Note that the separation is almost base-line which is important for a correct quantitative determination.

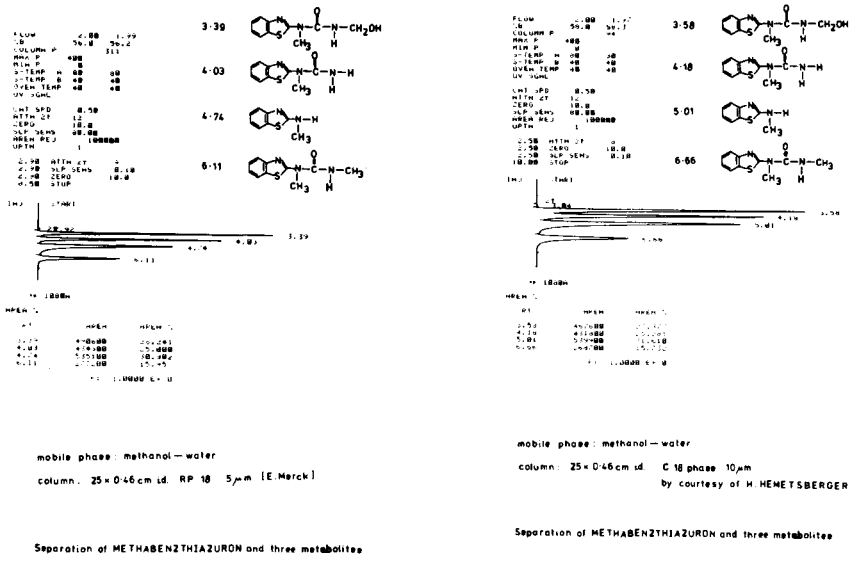

Fig. 2 Separation of methabenzthiazuron and its metabolites.

The 10 µm-material even showed a greater selectivity (!) than the 5 µm-material. An improvement should be achieved by using 5 µm-material instead of 10 µm-material.
Recently Führ et al. (7) also identified the major metabolite of MBT in soil as N'-methyl-N'-(2-benzothiazolyl)-urea (II).

Figures 3 and 4 show the separation of several herbicides in a single run. The simultaneous determination is important e.g. for monitoring river water or for a drinking water control.

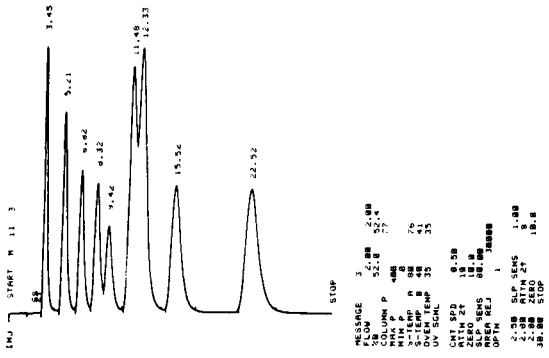

Figure 3

Figure 3
Column: material b) mobile Phase: Methanol-Water 52:48 (v/v)
elution order: Fenuron, Methoxuron, Monuron, Carbaryl, Monolinuron, Metobromuron, MBT, Diuron, Linuron

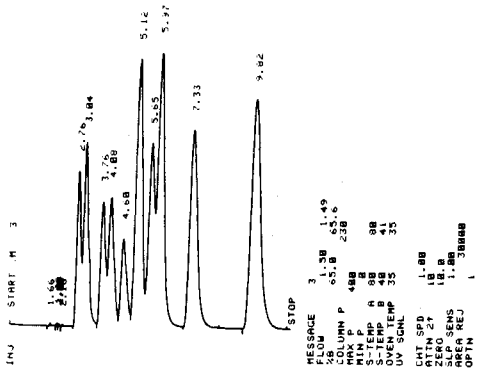

Figure 4
Column: material a) mobile phase: methanol-water 65:35 (v/v)
elution order: Same as above anly MBT and Diuron change their places

Thus high-performance liquid chromatography is shown as a powerful method for the determination of herbicides.

ACKNOWLEDGEMENT

The authors thank Dr. H. J. Jarczyk of Bayer AG (Leverkusen, F.R.G.) for supplying MBT and the metabolites as well as Dr. H. Hemetsberger of the Institute of Organic Chemistry II, University of Bochum (F.R.G.) for preparing and supplying of a stationary phase.

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Microanalysis of Aminotriazole by High Performance Liquid Chromatography

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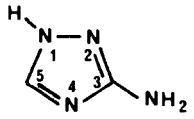
ABSTRACT

A widely used herbicide, 3-amino-1,2,4-triazole or amitrole, can be analysed by HPLC, after nitrosation at a pH comprised between 2.5 and 3 ; detection is made by UV spectroscopy at 254 nm ; the minimum quantity detected is 5 ng dissolved in 30µl, i.e. a concentration of 0,17µg.ml^{-1}. This method has been applied to detection and dosage of residual amitrole in soils and vine-leaves ; adapted extraction procedures are described.

KEY WORDS : 3-amino-1,2,4-triazole microanalysis by HPLC ; 3-amino-1,2,4-triazole nitrosation ; 3-amino-1,2,4-triazole residues in vineyard soils ; 3-amino-1,2,4-triazole residues in vineleaves.

INTRODUCTION

3-amino-1,2,4-triazole is a herbicide widely used in agriculture ; its acute toxicity is low-LD_{50} varies between 1 g and 25 g/kg of male rat according to authors - but carcinogenic properties, described by several authors (TSUDA, 1975 ; NAPALKOV, 1962 ; HODGE and co-workers, 1966 etc.) led to forbid its use in some countries, such as the U.S.A. So, it would be necessary to use an analytical method which would be sensitive, specific and quick, so as to detect any residue of the herbicide in every product proposed for human consumption. Several methods have been put forward to analyze aminotriazole : IR spectroscopy (GORE and co-workers, 1971), UV spectroscopy (WILLS, 1966), fluorimetry (MALLET and co-workers, 1971), paper chromatography (ALDRICH and co-workers, 1957 ; MITCHELL, 1960 ; STORHERR and co-workers, 1962) : all of them have drawbacks ; currently the best method is colorimetry of adducts obtained by action of various reagents on aminotriazole (GREEN and co-workers, 1957 ; AGRAWAL and co-workers, 1970 ; HERRETT and co-workers, 1962) ; the sensitivity of the best technique is fairly good (0,2 g.ml^{-1}), but the aliquot volume is 5 ml : so, the minimum quantity of herbicid in the analysed sample must be 1 g ; moreover, these colorimetric methods can lack specificity, because primary aromatic amines in the sample can give similar coloured derivatives : it is necessary to work up tedious purifications, susceptibl of favoring loss of product.



VPC is generally useful for microdetermination of pesticide residues, but it is very difficult to use with aminotriazole, as with many other nitrogen compounds, because it would be necessary to use special detectors, such as electrolytic conductivity detectors (MESTRE and co-workers, 1973 ; COULSON, 1966) or thermoionic detectors (HARTMAN, 1969), which are expensive and delicate to use.

These were the reasons why we tried to perfect an analytical method using high performance liquid chromatography (HPLC), which would provide opportunities of chromatographic techniques, without the disadvantages of VPC. UV absorptiometry is one of the most sensitive detection modes in HPLC ; aminotriazole itself having an absorption maximum below 200 nm, it is necessary to operate with one of its derivatives which absorbs in near UV. Such a derivative can be prepared by adding an excess of nitrous acid to an aqueous solution of aminotriazole acidified to pH 2.5-3 by acetic acid ; this derivative has an absorption maximum at 254 nm (ε = 4200) and a fair retention time in HPLC.

EXPERIMENTAL PART

I - HPLC microdetermination of aminotriazole :

a/ Nitrosation : about 3 ml of aqueous extract of aminotriazole provided by a suitable extraction of a known weight of material is introduced in a 5 ml standard flask ; add about 1 ml of washing water, then 0.15 ml of glacial acetic acid ; mix thoroughly ; add 0.5 ml of 5 % aqueous sodium nitrite solution ; fill to 5 ml with distilled water and leave for one hour before injecting.

b/ Standard solutions : prepare aqueous solutions containing amino-triazole from 0.25 $g.ml^{-1}$ to 5 $g.ml^{-1}$; nitrosation reaction is performed on each sample.

c/ Setting of HPLC instrument :

Apparatus	: Varian 8500
Column	: ID : 5 mm Length : 24 cm Packing : aminex A 7 cationic resin (SO_3^-) ; particle diameter from 7 to 11µ.
Delivery (output)	: 30 $ml.h^{-1}$
Elution agent	: aqueous sulfuric acid (pH = 4)
Detector	: UV (254 nm)
Reference	: aqueous sulfuric acid (pH = 4)
Chart speed	: 30 $cm.h^{-}$
Injection	: 10 to 30 µl.

d/ Experimentation : each standard solution of aminotriazole, nitrosated as indicated previously, is injected in the chromatograph ; the signal appears 13 minutes after injection. In each case, peak height is measured and related to the quantity of injected aminotriazole : the graph is linear. The solution to be analysed is injected ; the peak height is measured and the injected quantity of aminotriazole is deduced from the calibration curve ; the concentration of amino-triazole can then be computed in the analysed solution and in the initial sample.

II - Extraction methods :

1° / Extraction from clayey soil : prepare extraction solvent by mixing 120 ml of acetonitrile, 60 ml of water and 6 ml of 20 % aqueous ammonia. Weigh 100 g of earth in an erlenmeyer flask, add 60 ml of extraction solvent, close the flask tightly and mix thoroughly ; centrifuge ; the clear solution is recovered and the remaining earth is treated again by 60 ml of solvent, mixed and centrifugated ; repeat once again. The aqueous extracts are mixed, and ammonia is slowly boiled out ; the pH is then adjusted to 6, so as to flocculate the extracted clay ; filter, wash the filter with water, and concentrate under reduced pressure to a volume of about 3 ml with a rotative evaporator.

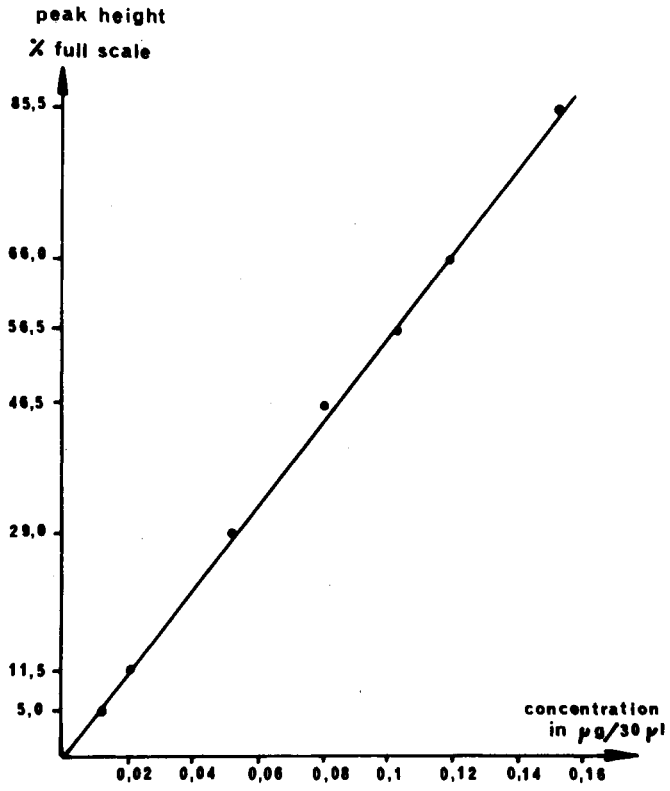
2° / Extraction from vine-leaves : about 25 g of leaves are weighed, finely cut, and crushed in a mortar ; a pinch of sand is added,and the mixture is ground to a paste : add 30 ml of acetonitrile, mix and decant the liquid paste ; repeate twice the last operation ; mix the extracts and centrifuge (10 mn at 4000 r.p.m.) ; the liquid phase is evaporated to dryness under reduced pressure (bath temperature below 65° C). The residue is treated three times by 5 ml of water and the aqueous solution is reduced to about 5 ml ; this extract is passed into a chromatographic column (10 mm I.D.) charged with a suspension of 3 g of polycar A.T. in water ; collect the eluted solution and add the washings of the column (10 ml of water) ; reduce to 3 ml in the rotative evaporator ; nitrosation is made on that extract.

CHARACTERISTICS OF THE METHOD

We verified, under the conditions we used, that the observed peak cannot be attributed to the reagents (sodium nitrite, acetic acid, aminotriazole) ; it is necessary to leave the mixture of reagents for one hour at room temperature to have a maximum intensity of the peak ; afterwards, this intensity remains constant for several days. The height of the peak is proportional to the concentration of aminotriazole ; a similar solution gives the same response whatever the time (> 1 h) between nitrosation and injection ; separate experiments of nitrosation on identic samples give the same results.

Detection limit of the method : the minimum quantity of aminotriazole giving a peak corresponding to 2.5 % of full scale (signal/noise $\simeq 5$) is 5 ng ; the maximum injection volume being 30µl it corresponds to a minimum detected concentration of $0.17 \mu g.ml^{-1}$.

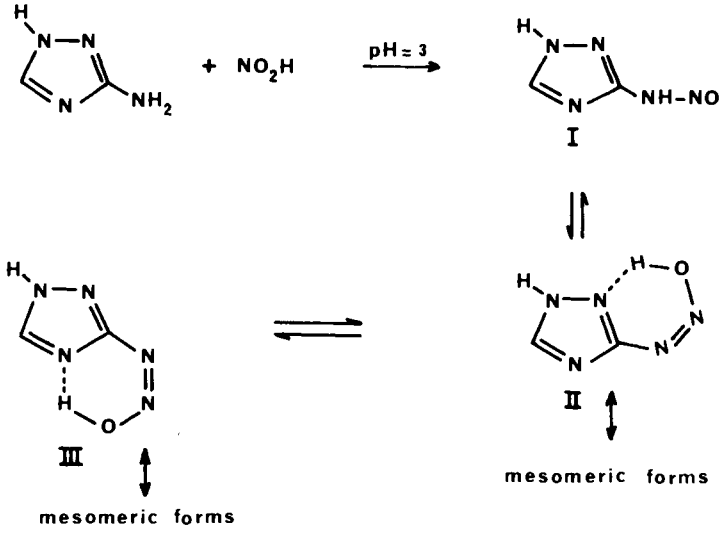
Accuracy : if the injected volume is small ($\leqslant 30\mu l$), the peak height can be considered proportional to its surface, and accuracy is $\pm$ 1 %.



Scheme I : CALIBRATION CURVE

Identification of the analysed derivative :

The nitrosated solution used for analysis does not give any colour with N(1-naphtyl) ethylene diamine ; conversely the diazonium salt of aminotriazole, obtained by nitrosation at pH 0, gives coloured reactions, but no peak in HPLC ; so the analysed compound is not a diaconium salt ; IR spectroscopy does not show N-H frequency at 3200-3400 cm^{-} : the amine function has been modified ; we believe that in a slightly acidic medium, the nitrous deamination reaction of the aromatic amine does not proceed to completion and is stopped at the level of a nitroso-derivative, specially if, after prototropy in hydroxydiazo form, it is stabilised by intramolecular H bond, as it is suggested by BUTLER and co-workers (1973) in similar cases.



CLOSSET and co-workers (1975) think that nitrous deamination of aminotriazole leads to 3-nitro 1,2,4-triazole, after substitution of the diazonium salt by nitrous ion ; we think the reaction path at pH 3 does not reach the diazonium salt step because we could not observe coloured reaction typical of such a compound with reagents like N(1-naphtyl) ethylenediamine ; furthermore, if a sample prepared for injection is brought to pH 0, a coloured reaction is observable, which could not be obtained from a nitroderivative.

APPLICATIONS OF THE METHOD

1° / Research of aminotriazole residues in soil :

We looked for residues of aminotriazole in vineyard soils near Perpignan (France), where weed control is usually carried out with this compound ; these soils contain much clay, and consequently we had to adapt the extraction procedure : works by ERCECOVIH and co-workers (1964), RUSSEL and co-workers (1968) shows that protonation and correlative fixation of aminotriazole in the soil depends on the nature of the cation(s) present in the clay. The recovery rate by the described method is 85 %.

Analysis of vineyard soils, weed controlled at the "usual" dose of 4,8 kg/ha showed that about 60 % of the herbicide remains after one week, 50 % after two weeks, 8 % after one month, 4 % after three months and 1 % after one year. We observed that remaining aminotriazole is always found in the upper part of the soil (5 cm) according to KAUFMANN and co-workers (1968) and RIEPMA (1962) : the decomposition of aminotriazole in soil could have a bacterial origin ; our results are in concordance with those of LEONARD and co-workers (1975) who found no more herbicide 21 months after spraying the soil at the dose of 16 pounds/acre (about 14,7 kg/ha, that is three times our dose).

2° / Research of aminotriazole residues in vine-leaves : we carried out an extraction procedure quicker than those previously described by ONLEY and co-workers (1969) and STORHERR and co-workers (1961), cumbersome pigments being eliminated by a method described by BOURZEIX and co-workers (1973) ; the recovery yield is 94 %.

Using the method described previously, we did not find any trace of aminotriazole in vine-leaves treated in the usual way (residue < 1 µg/100 g leaves) : an explanation may be that the herbicide may stay in the upper part of the soil, and cannot reach the roots of the plant. On the other hand, we experimented in laboratory on vines planted in sand and sprayed with an aqueous solution of aminotriazole at the normal dose, taking care not to wet the leaves ; nevertheless, we found after three months about 1 to 2 % of herbicide in the leaves.

3° / Other researches : we did not find any trace of aminotriazole in several samples of wine or grape-juice obtained from vineyards treated in the usual way : we could observe experimentally that about 50 % of herbicide added to grape-juice is destroyed during fermentation and wine making.

CONCLUSION

The analytical method described here is a little more sensitive than those previously published ; it is highly specific ; furthermore the experimentation is easy, quick, and less susceptible to errors or losses during handling.

Aminotriazole is almost completely destroyed at the surface of the soil in about one year, and in clayey soils it does not pass into vine-leaves ; that is not the case in sand. No residue of this herbicide could be found in wine or grape-juice coming from weed controlled vineyards.

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Chemical Carcinogenesis: A Natural and Man-made Global Environmental Problem

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ABSTRACT

One of the first environmental and occupational diseases of modern times is that of the chimney sweep's cancer of the scrotum which is produced by the carcinogenic hydrocarbons in the soot. At the beginning of this century tumours were experimentally produced by painting soot extracts on the skin of animals. Since then, extensive tests with different chemicals in animals have revealed that 25% of all the chemical compounds tested were found to be carcinogenic. In the light of the results of this extensive research and of the more recent findings on the presence of carcinogens in polluted urban air, drinking water, food, industrial and commercial products, a consensus has been reached by cancer investigators that 80-90% of all human malignancies can be ultimately traced to the effect of environmental agents. Some of these agents are physical and biological, but the majority of them are chemical compounds manufactured by man, either as primary or secondary products of our diverse human activities. Although our technological civilization may be principally responsible for the high incidence of this environmental disease, it should be noted that chemical carcinogens are also found among natural products. It is even probable that these compounds existed before life appeared on the Earth, as indicated by the polycyclic hydrocarbons detected in meteorites and in cosmic grains from supernovae explosions, as well as in ashes and lava from various volcanoes. It is also known that a number of living organisms, such as fungi (e.g., aspergillus) and plants (e.g. ferns and cycads) produce potent chemical carcinogens (e.g. aflatoxin and cycasin) which usually cause cancer and death to animals ingesting them.

A review of the most important naturally occurring and synthetic chemical carcinogens, and presently available methods for their detection will be presented.

INTRODUCTION

In this paper we are presenting information pertaining to the testing procedures and analyses of carcinogenic compounds in the environment as outlined in the following Table of Contents.

Testing Prodedures
- Bioassay
- Mutagenic

Compounds With Carcinogenic Activity
- Federal regulations in the United States
- OSHA list (PAH, alkylating agents, aromatic amines, nitroso compounds, natural occurring carcinogens)

Carcinogens in Air, Water and Food
- Carcinogens in air and their analyses
- Carcinogens in water and their analyses
- Carcinogens in food and their analyses

TESTING METHODS FOR CARCINOGENECITY

The relation between the incidence of scrotal cancer in chimney sweeps and their exposure to soot was recognized as early as 1775 by Percival Pott (1775). With the Industrial Revolution and the foundation of the chemical industry new carcinogenic substances were discovered, usually at the expense of many human lives. Until recently the majority of cancer causing agents were recognized after their effects on humans exposed to them became obvious (asbestos, vinylchloride). Today a considerable amount of chemicals are in constant use in industrialized countries and nothing is known about their carcinogenic activities. Thus it is a matter of survival to have reliable and fast methods to evaluate carcinogenecity of new chemicals and rigorous criteria of licensing before their release to the consumer. Such an undertaking requires reliable testing procedures which allows the unambiguous identification of carcinogenic compounds. It is obvious that the best results are obtained from epidemiological studies. This is documented by the incident of various types of cancers among workers exposed to industrial chemicals. The carcinogenecity of asbestos and vinyl chlorides has recently been established through such studies. However epidemiology alone cannot be the answer to the identification of carcinogenic chemicals. It is often difficult to obtain information from a group of people about the degree and period of exposure to a certain chemical because of job fluctuation, and also because of exposure to various other substances. In addition, for some chemicals the exposure period is extremely long, by the time its carcinogenic potential is known many lives are affected. Thus the regulation of carcinogenic substances requires methods by which the toxicity of these substances can be identified rapidly and unambiguously. Two different testing procedures are in use today: a long term test, the bioassay, which determines carcinogenicity, and a short term test based on bacterial studies, which examines the mutagenicity of the chemicals under investigation.

Bioassay

In the bioassay the test chemical is administered to small rodents in various ways, such as mixing with the food, skin painting, injection or through inhalation in the form of an aerosol. The treatment is continued generally over a period of two years or longer. In practice about 500 animals, including the controls, are needed to test one chemical. After the testing period the animals are sacrificed and abnormalities are examined by histopathological techniques. An examination of this kind will reveal

any changes that the tissue might have undergone, such as the presence of tumors, their cell origin and malignancy. The information obtained in such a bioassay is then used to characterize the carcinogenicity of the tested material.

The criticism most often expressed in relation to the bioassay method is that chemicals which are carcinogenic in test animals may not be necessarily carcinogenic in humans. It is argued that the mice or rats commonly used in these tests are inbred strains which are more likely to suffer spontaneous tumor formation. There is not much data available to prove or disprove this argument. However, in several cases, such as vinyl chloride and aflatoxin, the carcinogenicity of the chemicals was demonstrated in animal tests and later confirmed by epidemiological studies. The high dose levels which are administered to the test aminals are another source of controversy. The widely published case of saccharin is a recent example for such arguments. It is one of the inherent problems of carcinogenicity that some chemicals produce only a low incidence of cancer, or have an exposure period exceeding several times the life expectancy of the test animal. In order to maximize the detection of such chemicals they have to be administered in high doses. Although the controversy remains, the National Institute of Environmental Health Sciences maintains its position that substances carcinogenic in laboratory animals should also be regarded as carcinogenic in humans.

Bacterial tests

Because of the complexity of the bioassay and the long testing period, there is considerable interest in the development of short term systems which can identify chemicals with carcinogenic activity. The most often applied and probably most thoroughly studied short term test has been devised by Bruce Ames and is now generally known as the Ames test. The test uses certain mutants of the bacterium Salmonella typhimurium which have lost the ability to synthesize the amino acid histidine. In order to promote their growth the culture medium must contain the amino acid. If this strain is exposed to mutagenic chemicals, the bacteria regain their ability to grow in a histidine free medium. The interaction between the mutagenic chemical and the genetic material (DNA) is believed the triggering step in carcinogenesis. Thus mutagenic substances are also most likely carcinogenic. So far, impressive results have been obtained with the Ames test. It provided the first evidence that hair dyes and flame retardant chemicals in children's clothing (sleeping wear) may be potential carcinogens. More than 90% of carcinogenic substances proved to be mutagenic in a group of 3000 chemicals containing both carcinogenic and non-carcinogenic substances (Bridges, 1976; Purchase et al., 1976). Approximately 13% of the compounds tested positive although their carcinogenicity has not been established. The false-positive results, if proved to be false, are of much less importance than the false-negative results. A 90% correlation means that one out of 10 carcinogenic chemicals is missed by the test, a problem which has been discussed by several investigators (Bridges, 1976; Deserres, 1976; Purchase et al., 1976). It is known that many carcinogens must be changed in vivo to become oncogenic (Barry and Gutmann, 1973: Miller and Miller, 1974; Radomski et al., 1973: Scribner and Naimy, 1973). This activation is mainly done by oxygenases, found primarily in the liver and kidney, where a variety of compounds accumulate. It is believed that the lack of these enzymes in the cultured cells is the cause for errors in the Ames test. As a possible solution to this problem homogenates of liver cells have been added to the mutagenic cell culture. However the results obtained thus far are not reproducible. Other investigators suggested the parallel use of several standarized short term tests similar to the Ames test. A variety of such tests have been devised and are presently studied extensively (Maugh, 1978a; Stich et al., 1975).

COMPOUNDS WITH CARCINOGENIC ACTIVITY

Regulations

Although the mechanism which turns normal cells into cancer cells is still unknown for the most part, it is well recognized that certain chemicals can produce cancer. According to an estimate (Maugh, 1978b) there are 4.3 million different chemicals in existence, approximately some 63,000 are commonly used in the United States. Various efforts are now made by governmental agencies to compile an inventory of chemicals and to identify those substances which are hazardous. Federal legislation passed in the USA during the last eight years is aimed at regulating the level of toxic substances in the air, drinking water, food, as well as the exposure to these substances at the workplace and home. One of the most recent laws--The Toxic Substance Control Act (TSCA) will enable the U.S. government to prevent chemicals from distribution, or remove already existing chemicals from the market, if they are proven to be hazardous. Among the toxic chemicals prime attention is now given to carcinogens. These are some 2000 chemical substances which have been linked to carcinogenicity. The Occupational Safety and Health Administration (OSHA) which already regulates cancer causing chemicals at the workplace, has prepared a list of substances which are known to be carcinogenic or which are suspected carcinogens (Bingham, 1977; Chem. Eng. News, 1978). The chemicals are divided into four categories, based on the evidence of their carcinogenicity. Category 1 is comprised of 269substances (based on EPA data), whose carcinogenicity is unambiguously documented by clinical data from patient and mamalian test animals. The second category consists of 218 chemicals which are reported to cause cancer, but lack conclusive evidence. A remaining 396 chemicals, with no or only slight evidence of carcinogenicity are placed in Category 3.

Most of the carcinogens in Category 1 can be assigned to major groups or organic substances.

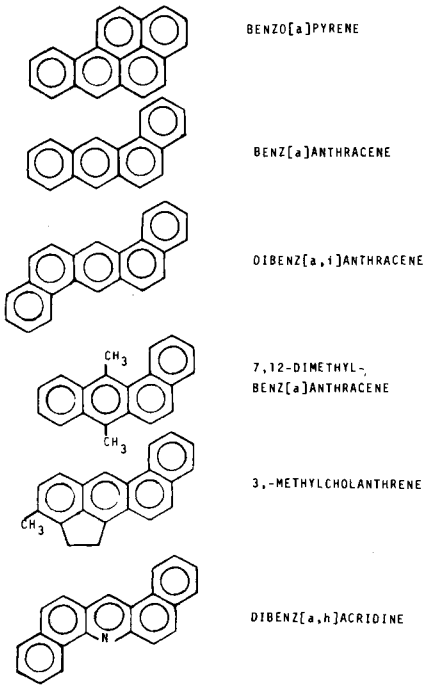


FIG. 1. CARCINOGENIC POLYCYCLIC AROMATIC COMPOUNDS

Polycyclic Aromatics

This class of compounds (Figure 1) includes aromatic hydrocarbons which have been related to cancer as far back as the 19th century. They are the main cause for skin cancer in workmen who handle tars or similar materials over a long period of time. Benzo[a]pyrene, a carcinogen found in tar, is also present in many consumer products. Formed via a pyrosynthetic pathway, benzo[a]pyrene has been detected in many consumer products. Formed via a pyrosynthetic pathway, benzo[a]pyrene has been detected in automobile exhaust gases, in tobacco smoke, roasted coffee, smoked food, such as sausage and fish, and in broiled hamburgers. Polycyclic aromatic hyrocarbons from untreated industrial waste and lubricating oil eventually find their way into the environment. Thus they have been identified in the air, as well as in drinking water.

Alkylating Reagents

Another large group of chemicals with carcinogenic potential are alkylating reagents and their precursors (Figure 2). The carcinogenicity is most likely based on their reactivity with nucleic acids and protein. Besides their abundance at the ppb-level in the air and drinking water, halogenated hydrocarbons have been used in large quantities in the dry cleaning industry and in the chemical industry. Vinylchloride, the starting material for many plastics, was recently recognized as a carcinogenic substance causing cancer of the liver in humans. EPA is now curtailing the production of vinylchloride and chlorinated hydrocarbons used as pesticides (DDT) and plasticizers (PCB's). Many chemicals, falling in the group of alkylating reagents have been associated with cancer of the liver, kidney and lung.

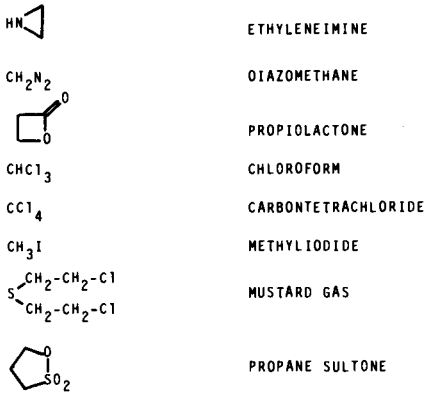


FIG. 2. CARCINOGENIC ALKYLATING REAGENTS

Aromatic Amines

Some carcinogenic aromatic amines are listed in Figure 3. Naphthylamine and its derivatives, as well as benzidine, are extremely potent carcinogens. Used in the dye industry they were the main cause for cancer of the bladder in workmen. Recently some food dyes which are azo-compounds were found to be unsafe.

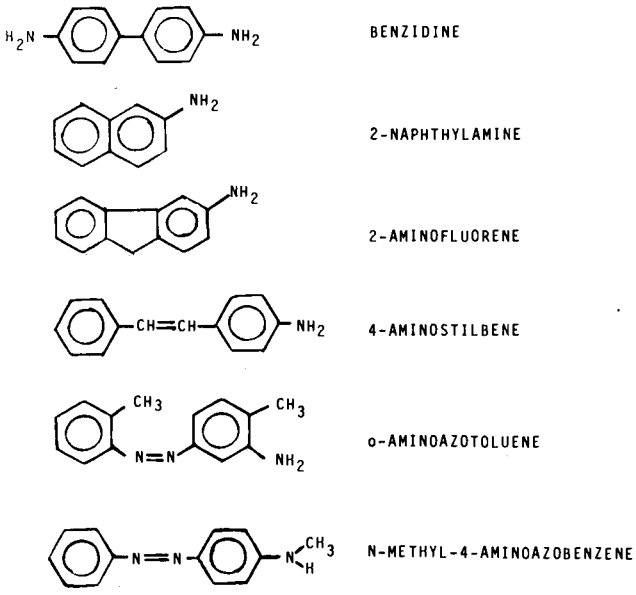


FIG. 3. CARCINOGENIC AROMATIC AMINES AND AZO COMPOUNDS

Nitroso-compounds

In Figure 4 some carcinogenic nitrosamines are listed. Nitrosamines are easily formed in a reaction between nitrous acid and secondary amines. They are powerful carcinogens and cause a variety of cancers, including cancer of the lung, liver, bladder, stomach, etc.

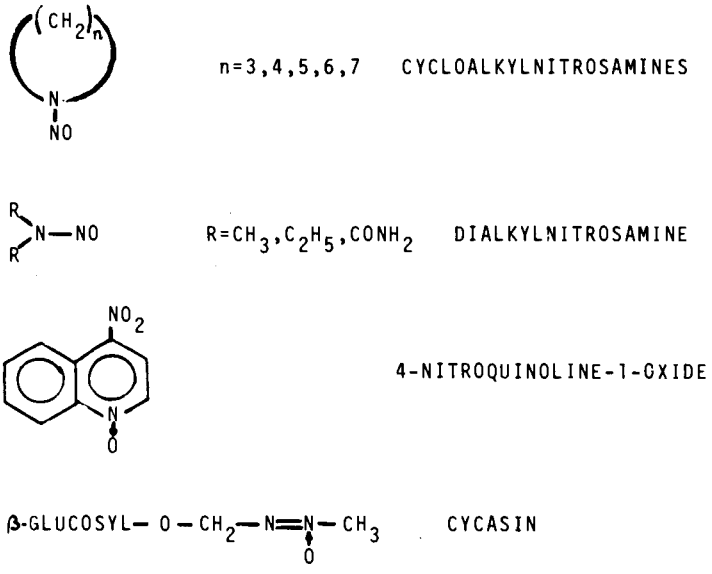
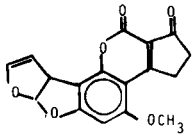


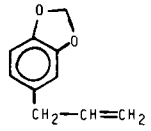
FIG.4. CARCINOGENIC NITOSO-COMPOUNDS

Carcinogens produced by plants or molds

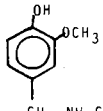
The compounds shown in Figure 5 possess various chemical structures and do not belong to a specific class of compounds. Some of these are present in the natural environment. Aflatoxin B_1, the most powerful carcinogen known, is produced by the yellow mold, Asperillus flavus. Recently guidelines have been established with respect to safe levels of this compound in contaminated peanuts. Chili pepper, especially favored in the southern United States, contains capsaicine, suspected to be a carcinogenic substance. Because of international trade activities many new food materials on the market, such as herbs, spices, herb teas or other exotic plant extracts need to be examined for their carcinogenicity.



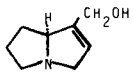
AFLATOXIN B_1



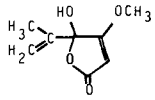
SAFROLE



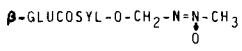
CAPSAICINE



SUPINIDINE



PENICILLIC ACID



CYCASIN

FIG. 5. CARCINOGENS PRODUCED BY PLANTS OR MOLDS

CARCINOGENIC COMPOUNDS IN AIR, WATER, FOOD AND THEIR ANALYSES

Carcinogenic Substances in Air

Polluted air (Table 1) affects the well being and health of all living things--man, animals and plants alike. The costs of property loss by soiling and deterioration caused by contaminants in the air is tremendous (Ridker, 1967). The accelerated decay of historical, architectural landmarks in Europe and America are the sad reminder of this development. Public awareness of these adverse effects of air pollution have now forced the legislatures in many countries to control the emission of pollutants from automobiles and smokestacks.

TABLE 1

SOURCES OF RECOGNIZED OR SUSPECTED CARCINOGENIC POLLUTANTS IN AIR

- NATURAL EMISSIONS FROM PLANTS AND TREES
  - HYDROCARBONS
- RELEASE OF VOLATILES FROM LARGE BODIES OF WATER (OCEANS, LAKES)
  - DDT IN ANTARCTIC ICE AND BODY FAT OF PENGUINS
  - PAH RELEASED INTO WATER BY NATURAL MECHANISMA OR OFFSHORE DRILLING ACTIVITIES
  - DUMPING OF WASTE CHEMICALS INTO THE OCEANS
- AUTOMOBILE EXHAUST GASES
  - NO_x, PEROXIDES, ALIPHATIC AND AROMATIC HYDROCARBONS
- INDUSTRIAL POLLUTANTS
  - ALL TYPES OF VOLATILE CHEMICALS
- POLLUTION FROM MUNICIPAL ACTIVITIES (HEAT GENERATION, POWER PLANTS, HUMAN SOCIAL HABITS)
  - SO_x, PAH, AROMATIC AND ALIPHATIC HYDROCARBONS, TOBACCO SMOKE

Environmental pollution has been linked for a long time with the diseases of the respiratory tract. Lung cancer is one of the most common causes of death in the industrialized countries. Epidemiological studies indicate tobacco smoking, air pollution and exposure to hazardous chemicals at the workplace as the main cause for the increased incidence of lung cancer.

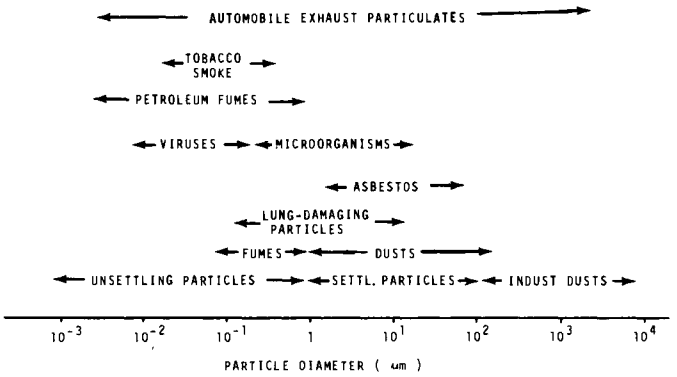
The effect of contaminants in the air on the incidence rate of lung cancer is difficult to determine because of the contribution of factors such as cigarette smoking and additional industrial exposure. The National Academy of Sciences estimated that the lung cancer is twice as common in persons living in urban areas Compared to those living in rural areas. In other studies a clear correlation between lung cancer and smoking habits could be established, however the death rates in smoking city dwellers was always higher than in the corresponding rural groups (Hammond and Horn, 1958a,b; Stocks and Campbell, 1955). Thus pollutants in the air seem to be the most likely reason for this observed increase in lung cancer. It is essential to determine the chemical nature of the carcinogenic substances as well as their concentration in air. There are two types of air pollutants. Particulate contaminants (aerosols) and organic or inorganic vapors (volatiles). Until recently the general approach of the analysis of pollutants in the air was the determination of compounds which have been recognized previously as harmful. Thus the vast majority of volatile materials in the air was left undetermined because of their unknown identity. In recent years a number of new carcinogenic contaminants in air were discovered (Hoffmann and Wynder, 1976), calling for a different strategy in the analysis of air pollutants. As a result efforts are now concentrated along these lines in the research facilities of the EPA in the US and in Europe under the authority of the Common Market countries (European Economic Community Cooperation and Coordination in the Field of Scientific and Technical Research, Action COST 66).

Particulate pollutants

The particulate air pollutants, which are responsible for the haze formation, have partial sizes ranging from 10^{-3} - 10^{2} μm Table 2). The major source of these contaminants are automobile exhaust gases and industrial waste products released into the atmosphere. These volatile chemicals undergo complex photochemical reactions with gases such as nitric oxides and with ozone, whose generation is directly related to automobile traffic. As a result highly reactive oxidants, such as peroxyacylnitrates (PAN) are formed. Under suitable atmospheric conditions (humidity, stagnant air masses) a mixture of various pollutants will form an aero-

sol, which is commonly described as smog, and with which so many of us are all too familiar.

TABLE 2 SIZE RANGES OF PARTICULATE AIR POLLUTANTS (after HOFFMANN AND WYNDER)



The analysis of the particulate material in the atmosphere involves three steps, sampling, separation and identification. Airborne particles are conventionally collected on glass fiber filters using standard Hi-Vol air samplers. A particle size discrimination--if desired--can be achieved by using samplers equipped with a cyclone. The material collected on the filter is then analyzed for its organic and inorganic content (Table 3).

TABLE 3

ANALYTICAL METHODS FOR THE ANALYSIS OF PARTICULATE AIR POLLUTANTS

1. LIGHT SCATTERING (PARTICLE SIZE)
2. EXTRACTION OF THE FILTERS
3. INFRARED SPECTROSCOPY (ORGANIC FUNCTIONAL GROUPS)
4. ULTRAVIOLET SPECTROSCOPY (POLYNUCLEAR AROMATIC HYDROCARBONS)
5. GAS CHROMATOGRAPHY-MASS SPECTROMETRY (VOLATILE ORGANICS)

The major elemental distribution is determined using optical emission spectrometry and X-ray fluoresence for the halogens, sulfur and lead. Wet chemical methods are generally applied for the determination of ions such as ammonium sulfate, chloride, nitrate and nitrite. For the organic analysis of the aerosol, the filters are subjected to Soxhlet extractions with solvents of different polarity, thus various subfractions are obtained. These fractions are usually bioassayed for tumorgenicity and carcinogenicity. Further separation of components in these fractions is achieved by chromatographic methods, such as liquid chromatography, thin layer chromatography and gas chromatography. Significant tumorgenic and carcinogenic activities are found in the aromatic hydrocarbon subfraction (Ashina et al., 1972: Hueper et al., 1962).

Detailed analysis of this group of compounds revealed the presence of carcinogenic polycyclic aromatic hydrocarbons in smog of all major U.S. cities (Hoffmann and Wynder, 1968; Sawicki, 1967). Some of these compounds are listed in Figure 1. Another group of substances in aerosol type pollution are polar substances such as aliphatic aldehydes, phenols, epoxides and peroxides, however they are only minor carcinogens.

Analysis of non-participating pollutants

Air quality measurement, as part of meteorlogical data collection is carried out today in all major U.S. cities. Among the permanent gases, which are routinely monitored by local air pollution control districts, nitrogen oxides have been

linked to cancer (Shapley, 1976). It is believed that they are involved in the formation of atmospheric nitrosamines, which are recognized as potent carcinogens. It is anticipated that NO_x, SO_x and O_3 undergo photochemical oxidation reactions with compounds such as aliphatic and aromatic hydrocarbons, which make up 90% of the organic pollutants in urban air. Thus ketones, aldehydes, lactanes, epoxides, peroxides, sulfonates, sulfones and nitroso-compounds have been isolated in urban air. Since these hazardous compounds are present at the ppb level, their identification and quantification demands a superior sampling system, as well as extremely sensitive instrumentation. Their low concentration excludes a direct analysis, therefore various methods for concentrating trace organics in air have been developed. In general, there are two sampling procedures. The cryogenic method (Altshuller et al., 1971; Bellar et al., 1963; Kaiser, 1973; Lonneman et al, 1968; Rohrschneider et al., 1971) is based on the trapping of organic volatiles of dry ice, acetone or liquid nitrogen temperatures. The technique is especially useful for highly volatile compounds. The major drawback however is aerosol formation and the excessive accumulation of water during the concentration. For example, a 100 liter air sample at room temperature and 50% humidity contains more than 1 g of water. Such high amounts of water present a tremendous problem during the gas chromatographic analysis of the concentrate.

The second method applied in the enrichment of trace organics in the air circumvents the water problem. The method is based on the dynamic adsorption of organics at room temperature onto a highly inert heat stable adsorbent, followed by thermal elution (Altshuller et al., 1966; 1971; Bellar et al., 1963; Bertsch et al 1974; Brooman and Edgerley, 1966, Hollis, 1966; Jones, 1966; Kaiser, 1973, Krumperman, 1972; Lonneman et al., 1968; Rasmussen, 1972; Raymond and Guiochon, 1974; Rohrschneider et al., 1971; Williams and Umstead, 1968; Zlatkis et al., 1973a). The performance of the system depends on the chemical nature of the adsorbent. To name a few requirements, an ideal adsorbent should have a

- high adsorption efficiency for organic compounds over a wide volatility range
- low affinity for water
- high recovery rates for all adsorbed organic compounds during thermal elution
- no decomposition or outgasing at high temperatures
- good storage properties of the sample without loss or change in the composition of the adsorbed organics
- compatibility to directly interface with the desired analytical system
- high reproducibility for quantitation

A vast number of adsorbents have been evaluated to their ability to retain organic compounds in air, but only a few meet the above requirements. Among those adsorbents, the most often applied is Tenax-GC, a porous polymer of 2,6-diphenyl-p-phenyleneoxide (Aue and Teli, 1971; Chintella et al., 1966; Dravnieks et al., 1971; Herbolsheimer, 1972; Herbolsheimer et al., 1972; Versino et al., 1974; Mieure and Dietrich, 1973; Saalfeld et al., 1971; Williams and Umstead, 1968; Zlatkis et al., 1973b; 1973c; 1974). It has become part of the sampling system now applied by EPA in the analysis of organic or inorganic volatiles in air.

The most efficient approach for the separation and identification of these organic volatiles is gas chromatography-mass spectrometry.

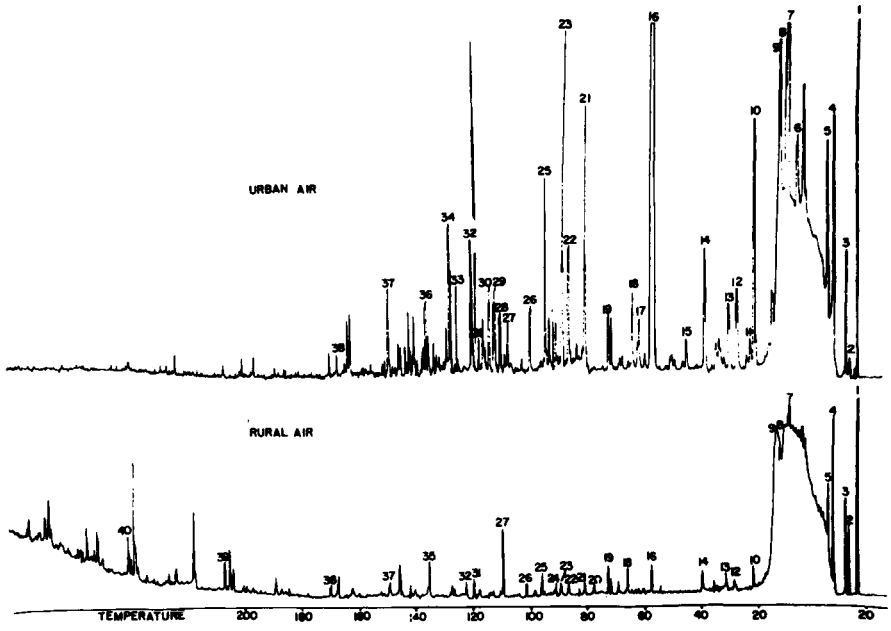


Fig. 6. Total ion current chromatograms (LKB 9000) of urban air (Tuscaloosa, Ala., U.S.A.) and rural air (Talladega National Forest, Ala., U.S.A.). Samples were collected in December 1976. Adsorbent, Tenax GC; sample size, 25 l; glass capillary column, 40 m × 0.35 mm I.D. coated with OV 101; carrier gas (helium) flow-rate, 3 ml/min; temperature program, 20° for 12 min, 20°–200° at 3°/min. For identification of the peaks, see Table 4.

TABLE 4

VOLATILES IDENTIFIED IN URBAN AIR AND RURAL AIR

No.	*Compound*	*No.*	*Compound*
1	Air*	21	C_8H_{16}
2	C_5H_{10}	22	Ethylbenzene
3	Acetaldehyde	23	*m,p*-Xylene
4	C_5H_{12}	24	Isoamyl acetate
5	Diethyl ether	25	Styrene
6	2-Methylpentane	26	Dimethylheptane
7	*n*-Hexane	27	α-Pinene
8	Chloroform	28	$C_{10}H_{20}$
9	Ethyl acetate	29	C_3-Alkylbenzene
10	Benzene	30	C_3-Alkylbenzene
11	Cyclohexane	31	β-Pinene
12	C_6H_{12}	32	$C_{10}H_{22}$
13	Trichloroethylene	33	1,3-Dichlorobenzene
14	*n*-Heptane	34	C_4-Alkylbenzene
15	2,4-Dimethylhexane	35	Limonene
16	Toluene	36	C_4-Alkylbenzene
17	2,5-Dimethylhexane	37	$C_{11}H_{24}$
18	Diethylcyclohexane	38	$C_{13}H_{26}$
19	*n*-Octane	39	$C_{14}H_{28}$
20	*n*-Butyl acetate	40	Benzophenone

* Introduced into the mass spectrometer by the sample introduction method.

The large amounts of data obtained in such an analysis have to be compiled by computers. In addition to data handling, library searches for unknown compounds can be carried out in order to identify them, or if this is not possible, a structural approximation of the unknown compound can be obtained. The method is of outstanding quality and only limited by the sampling procedure of the organic pollutants in air. A typical gas chromatographic profile or organic volatiles in urban air is shown in Figure 6. The compounds listed in Table 4 are typical organic air pollutants detected in most U.S. cities. Some of these substances are known or suspected carcinogens (Table 5), among them the group of polycyclic aromatic hydrocarbons (PAH), whose concentration is now constantly monitored by local air quality boards (Table 6).

TABLE 5

COMPOUNDS IN URBAN AIR WITH SUSPECTED OR RECOGNIZED MUTAGENIC OR CARCINOGENIC ACTIVITIES

SATURATED ALIPHATIC HYDROCARBONS	2-METHYLPENTANE
UNSATURATED ALIPHATIC HYDROCARBONS	METHYLPENTENE, METHYLBUTENE
AROMATIC HYDROCARBONS	BENZENE, ALKYLBENZENES, POLYNUCLEAR AROMATIC HYDROCARBONS (PAH)
ALDEHYDES	FORMALDEHYDE, ACETALDEHYDE, ACROLEIN
PEROXIDES	ACETYLPEROXIDE, PEROXYALKYLNITRATES
EPOXIDES	PROPYLENE OXIDES
NITROSAMINES	N-NITROSODIMETHYLAMINE
HALOGENATED HYDROCARBONS	VINYL CHLORIDE

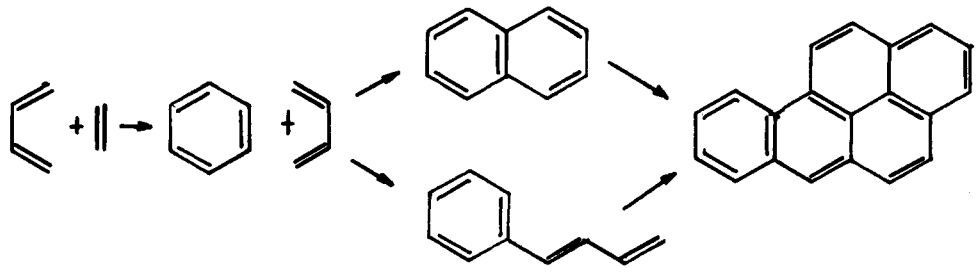
TABLE 6

CARCINOGENIC POLYNUCLEAR AROMATIC HYDROCARBONS IN URBAN AIR

(AFTER HOFFMAN AND WYNDER, 1976)

COMPOUND	$\mu g/1000m^3$	LOCATION
BENZO[a]PYRENE	0.05-74	U. S. CITIES (1958)
	54	LONDON (1968)
	5.0-18	SCANDINAVIAN CITIES (1968)
BENZO[b]FLUORANTHENE	2.7-6.5	U. S. CITIES (1958)
DIBENZ[a,h]ANTHRACENE	3.2-32	GERMAN CITIES (1966)
BENZ[a]ANTHRACENE	0.1-21.6	U. S. CITIES (1958)

Polycyclic aromatic hydrocarbons are formed via pyrosynthetic pathways (Badger, 1962), as outlined below.



The source and concentration of PAH's in urban atmospheres depends on the type of power generation plants, industrial activities and automobile traffic. It is estimated that the total PAH emission in the United States is 500 tons/year from heat and power generation, 600 tons/year from refuse burning, 200 tons/year from coke production and 20 tons/year from automobiles.

Carcinogenic substances in water

The increasing water demand in industrialized countries requires the utilization of water which has previously come into contact with man-made chemicals and effluents. (Table 7).

TABLE 7

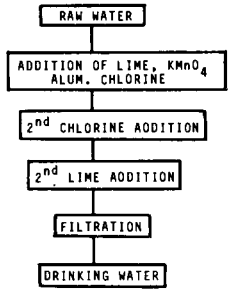
SOURCE OF ORGANIC MATERIALS IN WATER, UTILIZED AS DRINKING WATER

MATERIALS OF GEOLOGICAL ORIGIN:
HUMIC ACIDS, FULVIC ACIDS, INORGANIC MATERIALS
SUBSTANCES RELEASED INTO THE WATER SYSTEM BY EROSION OR RUNOFF
FERTILIZER, INSECTICIDES, PESTICIDES, SOIL CHEMICALS
ATMOSPHERIC FALLOUT
AIR POLLUTANTS, DELIBERATE OFFSHORE DUMPING OF CHEMICALS,
NATURAL RELEASE OF PETROLEUM INTO OCEANS
INDUSTRIAL POLLUTANTS AND MUNICIPAL SEWAGE FLUIDS:
ALL TYPES OF CHEMICALS, FIBERS (ASBESTOS)
HALOGENATED HYDROCARBONS FORMED DURING RAW WATER PURIFICATION

This drinking water is frequently obtained from polluted rivers and lakes and requires a multistep purification procedure (Table 8). Therefore it is important to monitor the quality of the drinking water constantly.

TABLE 8

SCHEMATIC DIAGRAM OF WATER PURIFICATION



It was recently shown that mixtures of residual organic compounds concentrated from municipal water supplies were mutagenic in the Ames test (Loper et al., 1977). Halogenated hydrocarbons have been discovered in drinking water of several U. S. cities (Dowty and Laseter, 1975; Dowty et al., 1975a; Dowty et al., 1975b), some of which may be carcinogens. These incidents focused much public attention on the water quality. It can be assumed that these substances have been in drinking water for some time, because we know now that they are formed at the ppb level during the chlorination process of the water treatment, a process which is in use in the United States since the beginning of this century. Chlorine is an effective disinfectant which controls bacterial population in water over extended periods of time. In water chlorine rapidly hydrolyzed to hypochlorous acid, which is the actual bacteriacidal agent. The production of organohalogens from organic

precursors is thought to proceed through a series of reaction steps in which hypochlorous acid acts as an electrophile (Carlson et al., 1975). Thus an exceedingly large number or organohalogens have been identified in drinking water (Table 9) (Bertsch et al., 1975), some of which--chloroform, carbon tetrachloride, trichloroethylene, tetrachloroethylene, dichlorobenzene--are suspected carcinogenic substances. The U. S. Environmental Protection Agency has recently proposed regulations to limit organic chemicals in potable water. A combined upper limit of 100 ppb has been suggested for the group of the so-called trihalomethanes.

TABLE 9

CONCENTRATION OF SOME SUSPECTED CARCINOGENIC ORGANOHALOGENS IN DRINKING WATER (µg/l)

COMPOUND	HIGH	LOW	AVERAGE
CHLOROFORM	5.5	4.4	4.6
BROMODICHLOROMETHANE	4.4	2.1	2.5
DIBROMOCHLOROMETHANE	0.012	0.007	0.01
BROMOFORM	1.1	0.85	0.94
BROMOCHLOROMETHANE	0.012	0.001	0.005
BROMOTRICHLOROMETHANE	0.02	0.002	0.006
CARBONTETRACHLORIDE	1.1	0.9	1.0
TRICHLOROETHYLENE	TRACE	TRACE	TRACE
TETRACHLOROETHYLENE	0.62	0.38	0.58
1,1,2,-TRICHLOROETHANE	1.1	0.76	0.99

The reason for the late discovery of these substances can be attributed to the lack of sensitive detectors and an appropriate sampling procedure. Since the electron capture detector is now standard equipment in gas chromatography and single ion monitoring improved the sensitivity in mass spectral techniques, the sampling procedure remains the limiting factor in the organic water analysis.

In the following some enrichment procedures of trace organics from water are described. The major ones can be classified according to the following principles:

a) Liquid-liquid partitioning. The organic substances are extracted with a solvent of low miscibility in a batchwise or continuous manner.

b) Liquid-solid adsorption. An inert gas is bubbled through the sample water and the volatile organic substances in the gas stream are cryogenically condensed or trapped at room temperature on an adsorbent. Regeneration of the volatiles is achieved by extraction or thermal desorption.

c) Liquid-solid adsorption. The sample is passed over an adsorbent. Regeneration of the organic substances is effected by elution with a solvent.

Besides these three methods, there are other approaches, such as membrane separation, freeze drying techniques and water distillation, however they have less significance in drinking water analysis. The main difficulty in drinking water analysis is the low concentration at which these substances are encountered.

The general requirements for the trace analysis of organics in drinking water have been discussed by Grob (Grob, 1973; Grob and Grob, 1974; Grob and Zurcher, 1976). It was concluded that the methods based on classical extraction and charcoal adsorption are unsuitable if large amounts of solvent and adsorbent were used.

Grob et al., (1975) introduced the micropentane extraction technique which avoids the solvent concentration step after extraction, thus eliminating an important source of error. Another method, introduced by Junk et al.(1974) has found widespread application. Water samples were passed over a large surface area polymer, which was then extracted. The procedure can be applied to most substances, regardless of their chemical nature and has been especially adapted to halomethanes (Kissinger and Fritz, 1976). Several authors have reported the use of polymers in conjunction with gas phase extraction, followed by thermal elution of the organic compounds (Bellar and Lichtenberg, 1974; Bergert et al., 1974: Kaiser, 1971; Lewin et al., 1972). With this procedure a gas is bubbled through the water. The organic substances which partition into the gas phase are trapped on a suitable absorbant at room temperature. Using thermal elution techniques, the substances are flushed on a gas chromatographic column for analysis. The stripping efficiency depends on the amount of solutes and also on the ions present in the water. Substances with high vapor pressure and low polarity are most readily removed from the water. Polar substances and substances with high molecular weight are amenable to analysis when the water temperature is raised during the stripping procedure (Bertsch et al. 1975; Grob, 1973).

In all described methods the final analysis of the organic substances is done by gas chromatographic methods, in conjunction with mass spectrometry for identification of the compounds. In the last few years significant advances have been made in the design and operation characteristics of the GC-MS instruments and this type of equipment has become more and more important in environmental trace analysis.

Carcinogenic substances in food

Since the discovery that skin cancer can be induced by aromatic hydrocarbons found in tar and coal fluids (Kennaway and Hieger, 1930) it was believed that carcinogenicity is somehow linked to man-made chemicals only. Apart from the possibility that some of these compounds can find their way into foodstuffs, it was recognized relatively late in the mid-50's that certain plants and microorganisms produce carcinogenic substances (Schoental, 1976). Since then much research has been done to study natural occurring carcinogenic substances and their presence in our diet (Table 10). One of the surprising finds was that food additives and food processing are related to carcinogenicity. Thus carcinogens in food can be divided into natural occurring carcinogenic agents (e.g.,mycotoxins, alkaloids), contaminants introduced during food processing (environmental pollutants, PAH and nitrosamine formation), and food additives (dyes, preservatives).

TABLE 10

SOURCE OF CARCINOGENIC SUBSTANCES IN FOOD

- CARCINOGENIC SUBSTANCES FORMED AS RESULT OF FOOD PROCESSING OR PREPARATION:
  - FORMATION OF PAH IN BARBECUED OR SMOKED MEAT
- CARCINOGENIC SUBSTANCES FORMED IN CHEMICAL REACTIONS IN FOOD:
  - NITROSAMINES
- CARCINOGENIC SUBSTANCES FORMED IN FOOD AS RESULT OF BACTERIAL ACTIVITIES:
  - MYCOTOXINS
- CARCINOGENIC SUBSTANCES ADDED TO FOOD:
  - FOOD DYES, SWEETENERS

Natural occurring carcinogens

Various mold metabolites (mycotoxins) have been widely recognized as potent carcinogens in animals (Ciegler and Lillehoj, 1968; Forgacs and Carll, 1962; Goldblatt, 1969; Wogan, 1965). The most widely studied compounds belong to the group of aflatoxins (Figure 5), the metabolites of the Aspergillus species (Goldblatt, 1969, Wogan et al., 1971). They have been recognized in turkey feed, after the loss of entire poultry farms in England (Lancaster, 1961). Subsequent research showed the presence of various aflatoxins in other farm animals and animal products used in the human diet (Allcroft and Carnaghan, 1963; Allcroft, 1969; Krogh et al., 1973; Hanssen and Jung, 1973; Holzapfel et al., 1966). Aflatoxin has been detected in peanuts (Lancaster, 1961; Pons and Franz, 1978), wheat, oats (Nagarajan et al., 1973; Sherertz et al., 1976), corn, rice (Shotwell et al., 1969), soybeans (Mislivec and Bruce, 1977; Shotwell et al., 1978), and cotton seeds (Marsh et al., 1969). Its carcinogenicity is well documented (Spensley, 1963; Wogan et al., 1974), e.g. it was demonstrated that doses of only 200 ng of aflatoxin per day causes cancer in rats (Wogan and Newberne, 1967). Aflatoxins have been linked with the incidence of liver cancer in many areas of Africa and Asia, where the climatic conditions favor the mold growth. A further example of contamination of foodstuffs by mycotoxins are the metabolites of the Penicillium species (Kinosita and Shikata, 1965). Luteoskyrin and a chlorine containing peptide produced by P. islandicum have been recognized as cancer causing substances in molded rice, commonly referred to as yellow rice (Wogan, 1969).

Various carcinogenic compounds have been isolated from plants. The pyrrolizidine alkaloids (Figure 5) which are found in the species of Senecia and Crotalarra (McLean, 1970; Bull et al., 1968) have been linked to liver lesions in test animals (Schoental and Magee, 1957; 1959) as well as in humans (Bull et al.,1968; Selzer and Parker, 1951). Members of the cycad group, which are used to prepare tea by certain South African tribes, are known to contain the carcinogenic substance, cycasin (Figure 5). The substance can induce tomors in laboratory animals (Hirono et al., 1968; Laqueur et al., 1963). Safrole, isolated from sassafras and capsaicin, found in chili peppers, are weak carcinogens (Homburger and Boger, 1968).

Food processing, food additives and carcinogenic substances

Carcinogenic polycyclic aromatic hydrocarbons (PAH) are present in the environment and might find their way to man and animal through their diet. Another possibility and perhaps more significant is the formation of PAH during food preparation. Smoked, charcoal broiled or barbecued meat products (Table 11) are found to have high levels of benzo[a]pyrene (Griciute, 1978; Howard and Fazio, 1969; Lijinsky and Shubik, 1965). Lijinsky and Roos(1967) demonstrated that the PAH's are formed during the pyrolysis of melted fat, dripping into the heat source. The introduction of PAH's was directly related to the closeness of the meat to the heat source nd to the fat content of the food. Interestingly, benzo[a]pyrene formation was not observed when the heat source was located above the meat. In addition to meat products benzo[a]pyrene was identified in coffee (Homburger and Borger, 1968) in vegetables (Grimmer and Hildebrandt, 1967; 1968)and frying oils (Homburger and Borger, 1968). The latter was shown to contain also a series of oxidized substances such as epoxides, peroxides and hydroxy compounds (Raulin, 1967; Fioriti et al., 1967) which have the potential to be carcinogenic.

TABLE 11
POLYCYCLIC AROMATIC HYDROCARBONS IN FOOD

FOOD	BENZO[a]PYRENE ppb	BENZ[a]ANTHRACENE ppb	REFERENCE
FRESH VEGETABLES	2.85-24.5	0.3-43.6	GRIMMER AND HILDEBRANDT, 1965
VEGETABLE OILS	0.4-14	0.8-1.1	BORNEFF AND FABIAN, 1966
MARGARINE	0.4-0.5	1.4-3.0	FRITZ, 1968
COFFEE	0.3-1.3	1.3-3.0	GRIMMER AND HILDEBRANDT, 1967
COOKED SAUSAGE	12.5-18.8	17.5-26.2	PREHN, 1971
SINGED MEAT	35-99	28-79	THORSTEINSSON AND THORDARSON, 1968
BROILED MEAT	0.17-0.63	0.2-0.4	GRIMMER AND HILDEBRANDT, 1968; LIJINSKY AND SHUBIK, 1965
BARBECUED RIBS	10.5	3.6	LIJINSKY AND SHUBIK, 1965

Nitrosamines comprise another group of carcinogenic substances which have been found in food. They obtained considerable attention after an epidemic food poisoning in Norway in 1962. Large number of sheep and cattle died after the consumption of nitrite-treated fish meal. Nitrates and nitrites are primarily added to food products to prevent bacterial growth and to preserve color and texture of food. It was demonstrated later that the toxic agents causing the liver necrosis in the animals were nitrosamines, probably formed during the processing of the fish meal (Ender et al., 1964; 1967). Other research confirmed the formation of nitrosamines from nitrite and secondary or tertiary amines (Mirvish, 1970; 1972), thus the possibility of a food contamination with these compounds may exist since the majority of meat products are treated with nitrite or nitrate (Fox and Nicholas, 1974; Lijinsky, 1976). Furthermore the in vivo formation of nitrosamines from nitrites under conditions similar to the human stomach are also likely to play a role (Pignatelli et al., 1976; Sinnhuber, 1970; Telling et al., 1976). Nitrosamines have been shown to occur in vegetable oils, cheese and milk products (Hedler and Marquardt, 1968) (Table 12). Nitrosopyrrolidine has been identified in friend bacon in levels up to 139 ppb (Havery et al., 1976) whereas raw bacon did not contain any nitrosamines. High concentrations of N-dimethylnitrosamine, N-nitrosopyrrolidine and N-nitrosopiperidine were found in spice cure mixtures ranging from 50-2000 ppb (Havery et al., 1976). If single packaging spices and curing salts were applied no nitrosamines could be detected. In the U.S. federal law prohibits the marketing now of meat spice cure mixtures other than in separated packings (William, 1973). Further more the U.S. Congress has recently taken steps to limit the use of nitrites as food additives. Other food additives, such as artificial sweeteners, are under investigation for possible carcinogenicity. Cyclamate has been removed from the U. S. market after it was shown to induce cancer of the bladder in mice (Price et al., 1970). It is however available in most European countries. There is still much controversy in the U. S. about saccharin, which is known to cause cancer in mice. The question whether the benefits of the sugar substitute outweight its possible carcinogenecity remains still unsolved. In the meantime warning labels appeared on soft drink cans containing saccharin.

TABLE 12 CARCINOGENIC NITROSAMINES IN FOOD

FOODSTUFF	CONCENTRATION ppb	REFERENCE
KIPPERS	40	ENDER AND CEH, 1967
SMOKED HADDOCK	15	ENDER AND CEH, 1967
BACON	1-6	ENDER AND CEH, 1967
DRY SAUSAGE	1-10	ENDER AND CEH, 1967
SALAMI	0.1-80	PREUSSMANN, 1978
FRESH FISH (U.K.)	1-9	PREUSSMANN, 1978
HERRING (HONGKONG)	5-100	PREUSSMANN, 1978
CHEESE	0-50	KROLLER, 1967

ANALYSIS OF CARCINOGENS IN FOOD

Aflatoxins

The qualitative and quantitative analysis of the four carcinogenic aflatoxins (B_1, B_2, G_1, G_2) has been carried out traditionally by thin layer chromatography (TLC) (Off. Meth.Anal.1975a). The sample is extracted with an organic solvent, usually chloroform and the extract is passed over silica gel columns. The partially purified effluent is then applied to TLC. Quantitation is done best by fluorodensitometric measurements (Beljaar and Fabry, 1972; Pons, 1971). The method is sensitive at the ppb level. The tolerable concentration range for total aflatoxin in peanuts has been set a 15 ppb (Schmidt, 1974). Recently high pressure chromatography (HPLC) has been applied for the analysis of aflatoxins (Pons and Franz, 1978; Pons, 1976). High recovery rates for aflatoxins, good reproducibility and sensitivity in the sub-ppb level are the advantages of the method (Takahashi, 1977).

Nitrosamines

Since it was demonstrated that nitrosamines are formed in reactions of nitrites with secondary or tertiary amines (Mirvish, 1970, 1972), analysis for nitrosamines should also include the determination of nitrite levels in food. Nitrosamines are conveniently analyzed by gas chromatography (GC). Their isolation from foodstuffs is effectively done by extraction techniques (Crosby et al., 1972, Howard et al., 1970; Mirvish, 1972; Sen et al., 1974). Using methanolic potassium hydroxide digestion, followed by liquid-liquid extraction with methylene chloride and further purification on silica gel columns, fourteen different nitrosamines could be separated and identified by gas chromatography-mass spectrometry (GC-MS). The method is sensitive to 10 ppb of nitrosamine in a variety of food products and has the potential to be applicable in the sub-ppb range if electron capture detectors are used.

The nitrates and nitrites in food products are generally analyzed by techniques involving nitration reactions (Off. Meth. Anal. 1975b) or diazotation followed by coupling with an aromatic amino or phenolic compound (Coppola et al., 1976). The reaction products are measured by colorimetric methods. The first technique is based on the colorimetric measurement of the yellow nitro-xylenol complex (Hänni, 1951) formed in the reaction between free nitrite and xylenol. A recent modification of the method uses GC to quantify the complex (Toyoda et al., 1978). In the second method the free nitrite is converted into the diazonium salt of sulfamic acid, which is then coupled to 1-naphthylamine. The absorption of the pink dye is measured at 525 nm. A variety of methods have been used for the extraction of nitrite from foodstuffs, they are reviewed by Fiddler and Fox(1978).

Polycyclic aromatic hydrocarbons (PAH)

A number of different methods have been developed for the analysis of PAH's. All techniques involve a combination of solvent extraction, column chromatography, UV-absorption, fluorescence spectroscopy and gas chromatography-mass spectrometry. In most investigations only benzo[a]pyrene is determined as representative PAH (Fritz 1971; Howard and Fazio, 1969; Tilgner and Daun, 1969), although other carcinogenic PAH's have been identified in food (Fritz, 1968; Howard et al., 1968; Lijinsky and Ross, 1967). The detection efficiency of the PAH's from protein rich food such as meat, fish and poultry, requires saponification of the ground sample, whereas oils and fats can be analyzed without this treatment. Saponification is conveniently done with methanolic potassium hydroxide. The PAH's are then concentrated by various liquid-liquid partitioning, using polar and non-polar solvents (Fritz, 1968; Haenni, 1968; Howard and Fazio, 1969; Howard et al., 1968; Malanoski et al., 1968; Tilgner and Daun, 1969). Further purification can be achieved by column chromatography on silica gel (Saito et al., 1978), or on Sephadex (Grimmer and Böhnke, 1975). The fractions obtained by this technique are examined by UV-absorption or fluorometry at the characteristic wavelengths. Final separation of the PAH's is achieved by paper chromatography (Mitchell and Banes, 1959), thin layer chromatography (Fritz, 1968; Toth, 1970) and by gas chromatography (Grimmer and Böhnke, 1975; Lao et al., 1975). The latter method is most attractive because of the simultaneous separation and quantitation of the PAH's and because of good reproducibility, high sensitivity and the potential for automation.

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N^6-(Methylnitroso)Adenosine: a Carcinogen of Environmental Significance

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ABSTRACT

The naturally-occurring nucleoside N^6-methyladenosine upon nitrosation, under conditions similar to those existing in the gastric contents, is converted into N^6-(methylnitroso)adenosine (m^6(NO)Ado), an effective multipotential carcinogen. This compound is also formed by tRNA nitrosation *in vitro*. We have studied several materials to determine the possibility that m^6(NO)Ado occurs in the environment. The analytical procedures were those used for volatile and nonvolatile N-nitroso compounds: high pressure liquid chromatography, thermal energy analysis, and mutagenesis assays. We have found that western-type human food and animal feces of animals fed with it contained a fraction of a N-nitroso compound with the same retention time as m^6(NO)Ado at concentrations ranging from 1.1-3.2 ppm. Therefore, m^6(NO)Ado can be considered as a carcinogen of possible environmental significance.

KEYWORDS

Carcinogenesis, mutagenesis, tRNA, nitrosation, N-nitrosaminopurines, food, feces analysis.

INTRODUCTION

The possibility that N-nitroso derivatives may be involved in the genesis of certain types of human cancer created the need for a rapid and reliable method for the determination of these types of compounds. The detection of cancer-inducing agents is usually carried out by epidemiological studies or by long-term carcinogenesis animal assays. A given carcinogen is generally uncovered after a sizable group of individuals have been exposed to its effects in a determined geographical area, occupation or life style. The need for a more rapid and sufficiently reliable detection system is evident because of the great number of new chemicals which every year are introduced in the environment; to determine the toxic and carcinogenic properties of all of these by epidemiological means or bioassay is practically impossible. In recent times, however, two new methods for the rapid and sufficiently accurate detection of chemical carcinogens have become available. The first consists in the applications of procedures of analytical chemistry to the isolation and characterization of a broad type of substances which possess potent carcinogenic properties, namely, N-nitroso compounds, by means of high pressure liquid chroma-

tography and thermal energy analysis (Fine and others, 1977). The second method, applicable to all kinds of chemicals and which requires very little equipment, is based on bacteriological assay techniques which can determine the degree of mutagenicity of a given chemical. All carcinogens tested thus far are mutagens; about 90% of those found mutagenic are carcinogenic. The ease of operation of this assay may compensate for the 10% gap in its accuracy.

N-Nitroso derivatives, along with aromatic polycyclic hydrocarbons and mycotoxins, constitute the main source of environmental carcinogens which are naturally occurring or result from the pervading industrialization (Lijinsky, 1976; Magee and others, 1976). N-Nitroso compounds have been detected in significant levels in a variety of sources, and the list of their occurrence is constantly growing: air, water, soil, food (mainly proteins), tobacco smoke, pesticides, cutting oils, cosmetics and pharmaceuticals (Fine and others, 1976; Issenberg, 1976). These N-nitroso compounds arise from the interaction of secondary and tertiary amines with nitrites which are excreted in saliva (in form of nitrate which is converted to nitrite by bacteria), intestinal tract, and occur in certain foods and in water. N-Nitroso compounds can be formed *in vivo* in the stomach or intestine by a reaction between nitrites and secondary or tertiary amines (Sander and others, 1968). The nitrosation of substituted amines is influenced by their basicity, the presence of catalysts such as potassium thiocyanate (usually found in saliva, with increased amounts in tobacco smokers (Boyland and Walker, 1974)), formaldehyde (present in smoke and smoked food), and phenols, such as those found in coffee. Most important for the rate of nitrosation, however, is the pH of the solution; for instance, a maximum of formation of dimethylnitrosamine (DMN) from dimethylamine was found at pH 3.4. Nitrosation at neutral and alkaline pH is also feasible (Keefer and Roller, 1973), especially in the presence of formaldehyde. Similar pH-dependence to that observed with aliphatic amines has been found by us for the nitrosation of monomethyl- and dimethylaminopurines (Giner-Sorolla and others, 1975).

A great variety of naturally-occurring, pharmaceutical, and pesticidal substances containing substituted amines have been studied as substrates in the nitrosation reaction with nitrites to determine the possibility of N-nitroso compound formation. A class of ubiquitous amines that has heretofore been overlooked includes the N-substituted amines in the hypermodified bases which occur in nucleic acids, mainly tRNA. Reaction of dietary and salivary nitrites with components of nucleic acids containing secondary amines during processing or in the gastric contents could result in the formation of potentially carcinogenic N-substituted nitrosamines (Giner-Sorolla and others, 1973; Giner-Sorolla and others, 1975; Giner-Sorolla and Taracido, 1975) (Fig. 1).

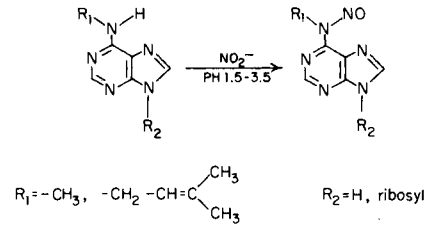

Fig. 1. Nitrosation of N^6-substituted purines

Among these nitrosamines is N^6-(methylnitroso)adenosine (m^6(NO)Ado) which has recently been demonstrated by Taylor (1978) to be formed by the *in vitro* nitrosation of tRNA. This nitrosamine could have a direct effect in the gastrointestinal tract or be absorbed through the intestine into the bloodstream and act on distant target organs.

We have assayed m^6(NO)Ado and found it to be a multipotential carcinogen in mice. After parenteral administration (30 mg total), up to 83% developed lymphomas and 93% had lung tumors. Oral administration of m^6(NO)Ado (60 mg total) also caused an increase in the incidence of lung tumors, and 80% of the females had mammary or reproductive tract tumors. In both cases the corresponding tumor incidence in the control animals was very low or nonexistent. The precursors of m^6(NO)Ado, namely, N^6-methyladenosine and nitrite given simultaneously to mice increased the lung tumor incidence over controls, indicating its formation in the gastrointestinal tract of the animal (Anderson and others, 1977). This finding has stimulated the investigation of the possible presence of m^6(NO)Ado in the feces of animals fed with the usual western diet, and to compare it with those obtained from regular laboratory Purina chow. The preliminary results of this work, the occurrence of m^6(NO)-Ado in food and animal feces is presented herewith.

ANALYTICAL METHODOLOGY

Physicochemical procedures. The methodology used in this work for the determination of the possible environmental significance of the carcinogen (m^6(NO)-Ado) consisted in the application of high pressure liquid chromatography (HPLC) and thermal energy analysis (TEA) for the detection and determination of low concentrations of this N-nitroso derivative. The thermal energy analyzer is an instrument specifically developed by Thermo Electron Corp., Waltham, Mass., USA, for the determination of N-nitroso compounds (Fine and others, 1976; 1977). The TEA detector depends on the thermal cleavage of the N-NO bond resulting in the release of a molecule of nitric oxide per molecule of compound introduced into the pyrolysis chamber. A schematic representation of the HPLC-TEA instrument is shown in Fig. 2 as it is used for the analysis of N-nitroso compounds (Fine and others, 1976, 1977).

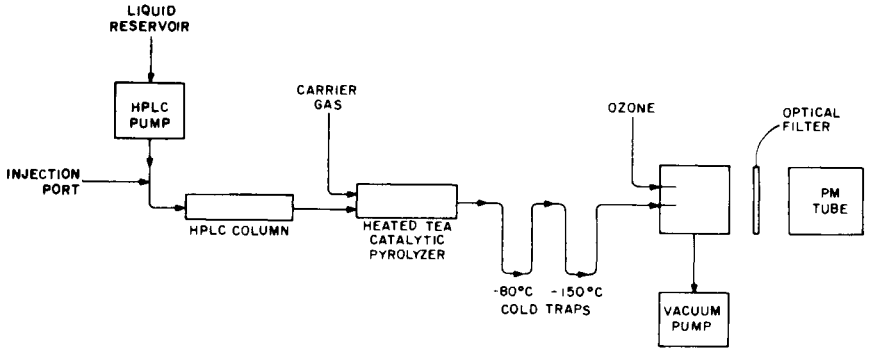

Fig. 2. Scheme of the HPLC-TEA interface

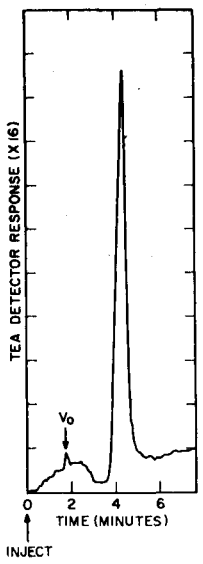

Fig. 3. HPLC-TEA chromatogram of m^6(NO)Ado standard (200 ng).

Chromatography and Detection. 10μ Lichrosorb Si60 analytical column 2-2.5 ml/min. Chloroform:methanol:acetic acid (300:20:5), Waters Associates Model 6000 A pump, Rheodyne HPLC injector. Thermal Energy Analyzer (TEA-502 Thermo Electron Corp., Waltham, Mass.).

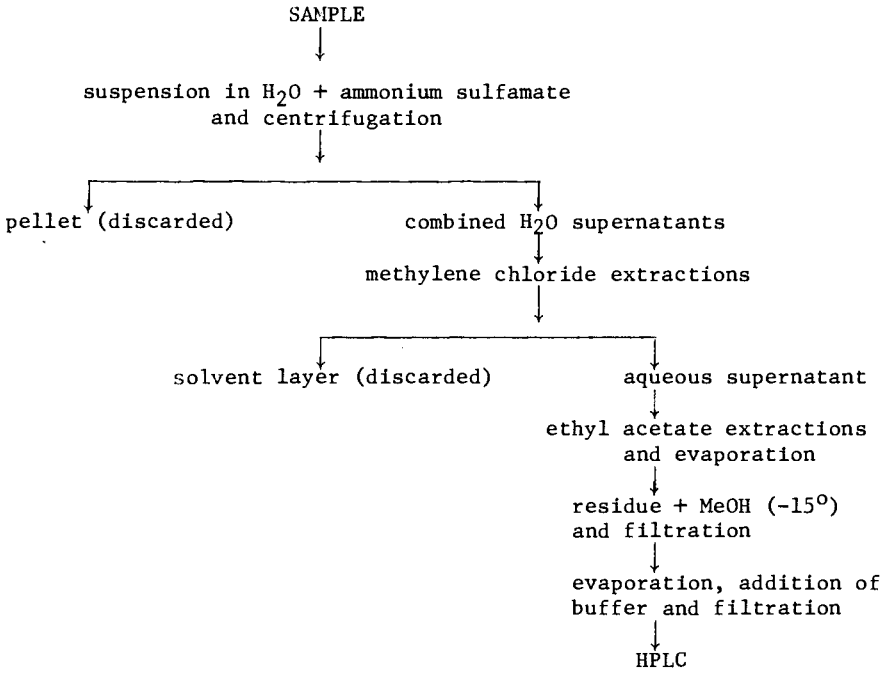

Fig. 4. Scheme for crude separation of N^6-(methylnitroso)adenosine

The liquid sample is swept through a catalytic pyrolyzer by the argon carrier gas, resulting in the generation of approximately one mole of nitric oxide, NO, per mole of N-nitroso compound originally present in the sample. The temperature used is about 400-500°. The effluent stream of the furnace is then passed through two cold traps at -80°/-150°. At this low temperature only the argon and nitric oxide and very few low molecular weight organic species pass through. The use of a cartridge filled with Tenax 6C (Applied Science Labs, State College, Pa.) can remove most of the organic molecules. The effluent then enters a reaction chamber kept at 1-2 mm Hg. The NO molecules are reacted with ozone generated *in situ*, resulting in the formation of excited nitrogen dioxide NO_2*. This excited NO_2* decays to its ground state with the concomitant emission of light in the infrared region of the spectrum. This light is then monitored with a photomultiplier tube, and the intensity of the emission is a direct measure of the amount of N-nitroso compound originally introduced into the TEA.

Bacteriological determination of mutagenesis. This assay is based on the property of mutagenic substances to induce revertants to the original wild strain from especially modified *Salmonella typhimurium* bacteria. The test was developed by Ames (1973) and is used to identify mutagens and carcinogens (McCann and others, 1975). It is a relatively simple procedure, about 90% accurate, to determine the mutagenicity-carcinogenicity of a chemical.

RESULTS

High Pressure Liquid Chromatography and Thermal Energy Analysis (HPLC-TEA)

The animals used were adult CF rats fed with common western diet including nitrite-preserved meats, smoked fish, green vegetables, wheat germ, borscht, beer, instant coffee, and tap water. Control animals (blank assays) were fed the usual Laboratory Purina Chow. The eaten food was prepared by mixing the diet components (obtained in form of a cake heated at 200° for 1 hr) with human saliva at 37° for 30 min, then treated with 0.1 N HCl at 37° for 2 hr.

In our preliminary work for the determination of m^6(NO)Ado in food and feces, the material was extracted by the following procedure: the sample (about 5 g) was mixed with ammonium sulfamate and homogenized in S-water (reagent grade Milli-Q2 system, Millipore Corp.). The homogeneous suspension was extracted 5 times with 3 volumes of ethyl acetate. The combined ethyl acetate extracts were filtered, treated with anhydrous sodium sulfate and evaporated to dryness under reduced pressure. Methanol was added to the residue, the mixture was frozen and then filtered. The filtrate was evaporated under reduced pressure at 20°, the residue taken up with phosphate buffer (6 mM, KH_2PO_4, pH 6.5), and this solution was used for HPLC-TEA. A standard curve of m^6(NO)Ado was obtained by using 200 ng in the following operational HPLC conditions: 5μ lichrosorb column with chloroform:methanol:acetic acid (300:20:5) at 1 ml/min flow rate, which is shown on Fig. 3. The minimum detectable amount of m^6(NO)-Ado was 1-5 ng of analytically pure material. A signal-to-noise ratio of at least 3:1 as the limits of sensitivity or minimum detectable levels was used in this work. Values lower than 3:1 are not considered reliable. A modified extraction procedure involving the centrifugation of the material suspended in S-water and subsequent treatment of the supernatant with methylene chloride as shown in Fig. 4 was alternatively used.

		m^6(NO)Ado ppm*
Sample 1:	Food extracts	
	direct	1.1
	spiked	2.2
	blank	<0.4
Sample 2:	Eaten food	
	direct	<0.5
	spiked	4.8
	blank	<0.4
Sample 3:	Fecal extracts	
	direct	3.2
	spiked	7.6
	blank	<0.4

*The expressed ppm are those of a fraction corresponding to a substance having the same retention time as m^6(NO)Ado.

Fig. 5. Results of HPLC-TEA analysis

Mutagenesis Determination

N^6-(Methylnitroso)adenosine (m^6(NO)Ado) was shown to mediate the back mutation of several strains of *Salmonella typhimurium* histidine-requiring mutants supplied by Dr. B. N. Ames. Without metabolic activation, m^6(NO)Ado was shown to effect base-pair substitutions as indicated by mutation of strains TA100 and TA1535. Frameshift mutation detector strains were not sensitive to m^6-(NO)Ado.

The assays were carried out in petri plates containing an underlayer of minimal-glucose agar. This was overlaid with agar containing 0.5 mM L-histidine and biotin. Spot tests were performed by placing a filter paper disc containing a known amount of m^6(NO)Ado in dimethylsulfoxide (not mutagenic at concentrations used in this assay) on hardened top agar into which a given *Salmonella* tester strain had been incorporated. Of all strains tested, only TA100 (a more sensitive strain derived from TA1535) gave a positive spot test with m^6(NO)Ado (~800 µg). Plate incorporation tests were carried out with m^6(NO)Ado added into the top agar-bacteria suspension. The number of test-specific mutants (observed mutants minus spontaneous mutants) were directly proportional to m^6(NO)Ado concentration in the range ~150-3500 µg/plate. Above this concentration the colonies were too numerous to count, with no cytotoxicity at 7 mg/plate. Using TA100, it was shown that m^6(NO)Ado was only about 1000-fold less active than the potent mutagen N-methyl-N'-nitro-N-nitrosoguanidine, a control base-pair substitution mutagen which does not require metabolic activation.

DISCUSSION AND CONCLUSIONS

The mounting evidence that N-nitroso compounds derived from nitrosation reactions of substituted amines may constitute one of the most significant sources of carcinogens in the environment has stimulated the development of reliable and sensitive techniques. Unlike carcinogens whose occurrence is limited to the working place in industrial areas, polluted urban zones or several lifestyles, carcinogenic nitrosamines from nucleic acid components

could arise from daily exposure of whole populations to the ingestion of usual foodstuffs.

Although the amount of methylaminopurines in food may be very small, the ubiquitous presence of nucleic acids from dietary sources and nitrites from salivary, intestinal, dietary and environmental origin and the ease of their interaction in the gastrointestinal tract, specially in the presence of the mentioned catalysts, could result in the frequent endogenous generation of the carcinogenic m^6(NO)Ado. This agent could act *in situ* or be absorbed in the intestine and interact with target organs. The recent finding by Taylor (1978) that certain types of tRNA upon nitrosation *in vitro* results in the formation of m^6(NO)Ado lends support to this assumption.

We tried initially to determine the amount of m^6(NO)Ado by several colorimetric procedures; the methods from the literature lacked sensitivity and frequently gave false positives. The HPLC-TEA system has a great sensitivity and a more reduced possibility of false positives. We shall have to collect sufficient material for the confirmation by mass spectrum of the results obtained by the HPLC-TEA determinations. The mutagenicity data obtained from the HPLC fractions by the Ames test will also be indicative albeit not confirmatory of the presence of m^6(NO)Ado in foodstuffs or in animal feces.

As a result of epidemiological studies and bioassays carried out in recent decades, it has been postulated that about 90% of all human cancers are attributable to chemical agents of endogenous or environmental origin. The ever-increasing industrialization and urbanization of our planet and their relationship to the growing incidence of certain types of human cancer has stimulated an intensification of the monitoring of the environment. Our research is directed toward the uncovering by analytical procedures and animal assays of a new source of carcinogens and to find, by appropriate and simple prophylactic dietary measures, the prevention of their effects.

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Gas Chromatographic - Mass Spectrometric Studies on Styrene Toxicity

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ABSTRACT

A series of analytical techniques are described which may give a deeper insight into the mechanisms of styrene toxicity. These techniques include: ultraviolet (UV) photometry, thin layer chromatography (TLC), liquid scintillation counting (LSC), gas liquid chromatography (GLC), mass spectrometry (MS), mass fragmentography (MF) and mass chromatography (MC). Styrene, styrene-7,8-oxide, reduced glutathione and the activities of toxifying (styrene-7,8-oxide forming monooxygenase) and detoxifying (styrene-7,8-oxide hydrase and glutathione-S-styrene-7,8-oxide transferase) enzymes were assayed in the liver, brain, lungs, heart, perirenal fat, spleen and kidneys of male mice with a view to building an experimental model able to predict which body districts are most likely to be targets for styrene toxicity. The results described show a good correlation between our in vitro and in vivo data and reports in the literature concerning the in vivo toxicity of this vinyl compound towards discrete tissues in man and laboratory animals.

New perspectives on styrene toxicity are opened by the identification in rat urine of 4-vinylphenol, p-hydroxymandelic acid, p-hydroxybenzoic acid and p-hydroxyhippuric acid. This ancillary metabolic pathway leading to the formation of phenolic type metabolites has been seen to result from chemical rearrangements of unstable arene oxides, reactive species which may be implicated in styrene toxicity.

KEYWORDS: styrene, metabolism, toxicity, phenolic metabolites, arene oxides, styrene-7,8-oxide forming monooxygenase, styrene-7,8-oxide hydrase, glutathione-S-styrene-7,8-oxide transferase, analytical techniques.

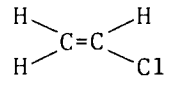
INTRODUCTION

Today's interest in the analysis and measurement of organic and inorganic environmental compounds is centered on the fact that many of them have toxic effects in man, ranging from relatively simple allergic manifestations to severe tumoral forms. A large number of these compounds are toxic only after biotransformation into "reactive intermediates".

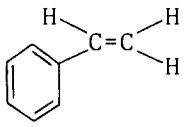
The search for an explanation of the carcinogenic, mutagenic and some other forms of toxicity in which reactive intermediates might be implicated, must take three main factors into consideration: i) the rate of formation of these electrophilic species, ii) their reactivity towards nucleophilic agents such as DNA, RNA and proteins, and iii) their chemical and enzymatic stability to reach target sites.

In the light of these observations it is important to identify all possible forms of reactive species in the metabolism of these compounds even if they only count as products of ancillary pathways, and to characterize the finer biochemical mechanisms which might be related with certain types of toxicity. This information could help us predict which body districts are likely to become targets and could also provide the means for modulating the toxicity by modifying the rate of metabolism (i.e., effects of inducers and inhibitors).

During the last few years, we have studied the biochemical factors at the basis of the toxicity of a class of vinyl compounds, including vinylchloride (I), styrene (II), vinylacetate (III) etc. (Belvedere and others, 1976, 1977; Cantoni and others, 1978; Pantarotto and others, 1978; Pantarotto and Bidoli, 1979a; Salmona and others, 1976; Zuccato and others, 1979).



(I)



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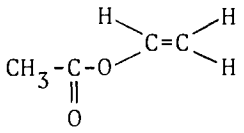

(III)

As an example, we will take styrene, one of the most widely used raw materials in the modern polymer industry and for which "long term carcinogenesis studies" are still in progress. To give an idea of the importance of this compound it is sufficient to say that, in the world, its production amounts today to about 7 million tons per year.

The metabolism of styrene is well known and is shown in Fig. 1.

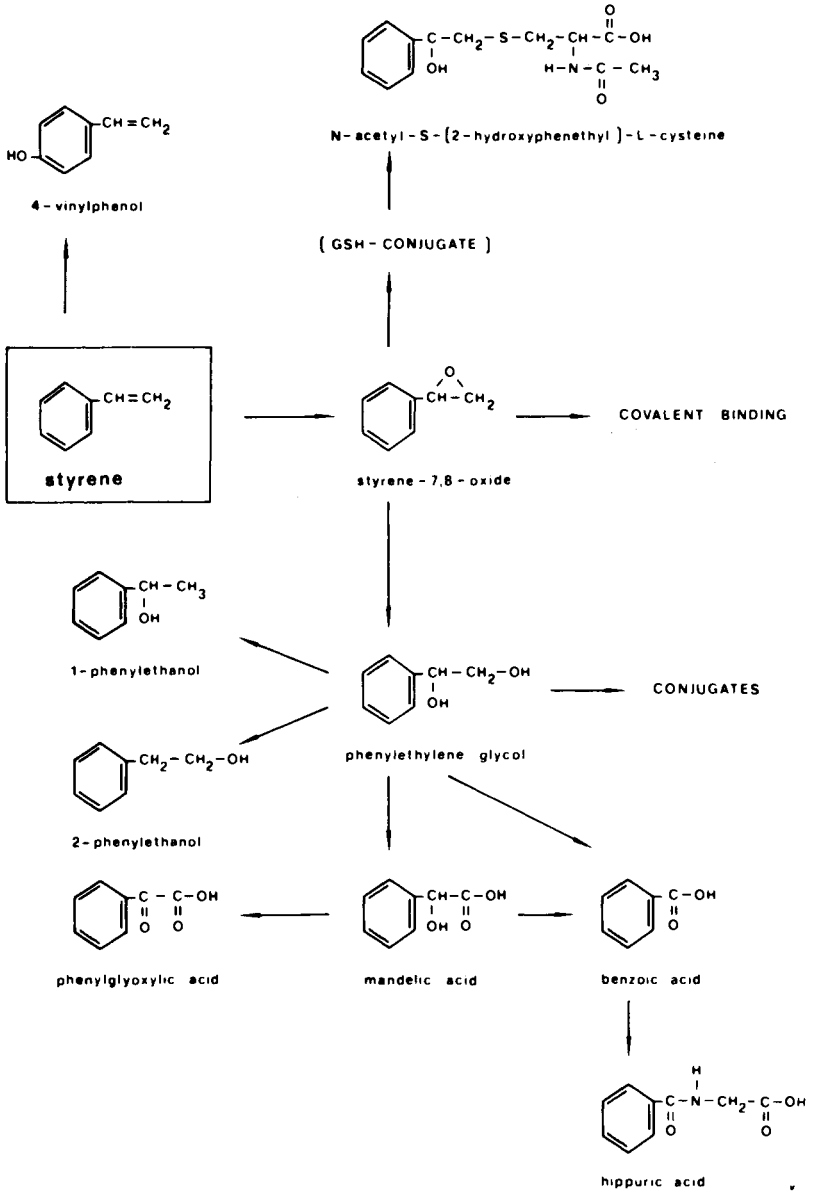

Fig. 1. Metabolism of styrene

It is now generally accepted that styrene toxicity may be ascribed to styrene biotransformation by epoxide forming monooxygenases into the more reactive species styrene-7,8-oxide, a compound known to be mutagenic, which covalently binds to rat liver macromolecules both in vitro and in vivo (Boyland and Sims, 1960; De Meester and others, 1977; El Masri, Smith and Williams, 1958; Fleig and Thiess, 1978; Holmberg, 1977; James and White, 1967; Leibman and Ortiz, 1969, 1970; Linnainmaa, Meretoja and Sorsa, 1978; Loprieno and others, 1976, 1978; Marniemi and others, 1977; Meretoja and others, 1977, 1978; Meretoja, Vainio and Järventaus, 1978; Milvy and Garro, 1976; Pagano and others, 1978; Vainio and others, 1976; Watabe and others, 1978a,b). Detoxification steps are styrene-7,8-oxide hydration to phenylethylene glycol with subsequent oxidation to mandelic, phenylglyoxylic, benzoic and hippuric acids, and styrene-7,8-oxide chemical and enzymatic glutathione conjugation with urinary elimination of mercapturic acids (Bardodej, 1964, 1978; Bardodej and Bardodejova, 1970; Bardodej, Bardodejova and Gut, 1971; Boyland and Williams, 1965; Härkönen and others, 1974; Ikeda and others, 1974; Ohtsuji and Ikeda, 1971; Seutter-Berlage and others, 1978). A minor metabolism of styrene to 1- and 2-phenylethanols and to 4-vinylphenol has also been described (Bakke and Scheline, 1970).

Thus the in vivo styrene toxicity in any body district, represented in Fig. 2 as the result of a covalent binding between the reactive species styrene-7,8-oxide and a cellular macromolecule, is closely related to biotransformation of this vinyl compound by different tissues.

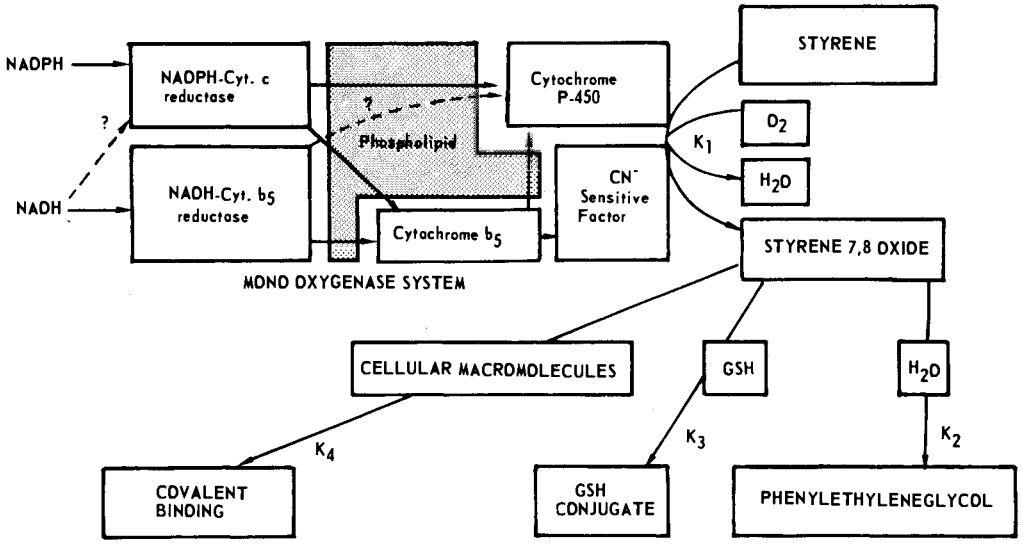

Fig. 2. Biochemical mechanisms of styrene toxicity related to its biotransformation to the 7,8-oxide metabolite.

This paper describes a number of analytical methods by which we correlated styrene levels and the ratio of toxifying to detoxifying enzymatic activities with a view to obtaining an experimental model for toxicity studies. These techniques also open new perspectives on styrene toxicity, through identification of phenolic metabolites in the rat (Pantarotto and others, 1978). This minor metabolism is interesting because phenolic compounds may be formed as a result of chemical rearrangement of unstable arene oxides as shown in Fig. 3.

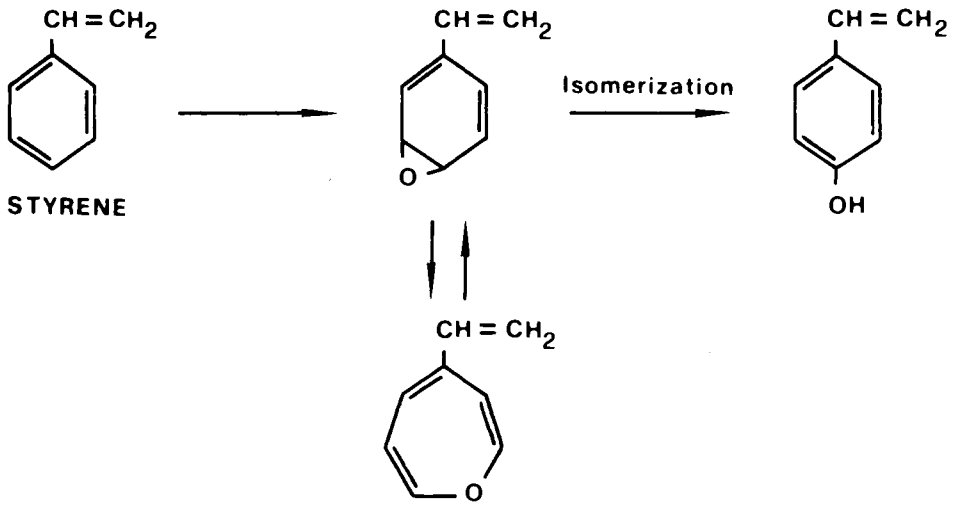

Fig. 3. Isomerization of styrene-3,4-oxide to 4-vinylphenol.

MATERIALS AND METHODS

Chemicals

Styrene, styrene-7,8-oxide, phenylethylene glycol, mandelic acid and hippuric acid were supplied by Merck, Darmstadt, West Germany. Phenylglyoxylic acid, p-hydroxymandelic acid and p-hydroxybenzoic acid were obtained from EGA Chemie, Steinheim/Albuch, West Germany.

^{14}C-Styrene (specific activity, 0.5 mCi/m mol, uniformly labelled at the 8 position) was purchased from NEN, Boston, Mass., USA; ^{14}C-phenylethylene glycol was prepared enzymatically, incubating the labelled styrene with rat liver microsomes. The specific activity of this labelled compound, determined by LSC and by GLC, was 0.5 mCi/m mol (Pantarotto and others, 1978).

Phenobarbital was obtained from Merck, Darmstadt, West Germany, and 3-methylcholanthrene from Sigma, St.Louis, Mo., USA. All reagents used were of analytical grade.

Animals

Male CD_2F_1 mice (20-22 g body weight) and male Sprague Dawley rats (200-220 g body weight) were obtained from Charles River Company, Calco, Como, Italy.

Liver microsomal cytochrome P-450/448 was induced with phenobarbital (80 mg/kg intraperitoneally in 0.9% saline twice a day for three days) or with 3-methylcholanthrene (40 mg/kg intraperitoneally in corn oil once daily for three days).

Mice for kinetic and metabolism studies were given a single intraperitoneal injection of styrene (200 mg/kg dissolved in corn oil). Groups of mice were killed by decapitation at different intervals thereafter and blood and tissue samples (liver, brain, lungs, heart, perirenal fat, spleen and kidneys) were collected, homogenized in phosphate buffer pH 7.4 to a volume of 3 ml and stored at -20°C in sealed hypo vials (6 ml volume) until required for analysis. Urine samples were collected after 48 hours from groups of animals housed in metabolism cages.

Rats for metabolism studies were housed in individual metabolism cages with water only. Styrene was injected intraperitoneally as a single dose (200 mg/kg dissolved in corn oil) and urine samples were collected after 48 hours. In other experiments rats were given ^{14}C-styrene diluted to a specific activity of 25 μCi/m mol.

GLC-MF Determination of Styrene

Styrene was assayed in biological specimens by a head space GLC-MF procedure described by Pantarotto, Fanelli and Bidoli (1979). A scheme of the method is reported in Fig. 4. The compound was quantitatively evaluated using ethylbenzene as internal standard. The sensitivity of the method depends on the type of detector used, flame ionization detector (FID) or MF.

GLC-MF Determination of Styrene-7,8-oxide

Styrene-7,8-oxide was assayed in biological material by GLC-MF after micro-extraction with methylene chloride according to Pantarotto and Bidoli (1979b). A scheme of this procedure is shown in Fig. 5. The compound was quantitatively evaluated using p-methylanisole as the internal standard. The sensitivity of this method depends on the type of detector used (FID or MF).

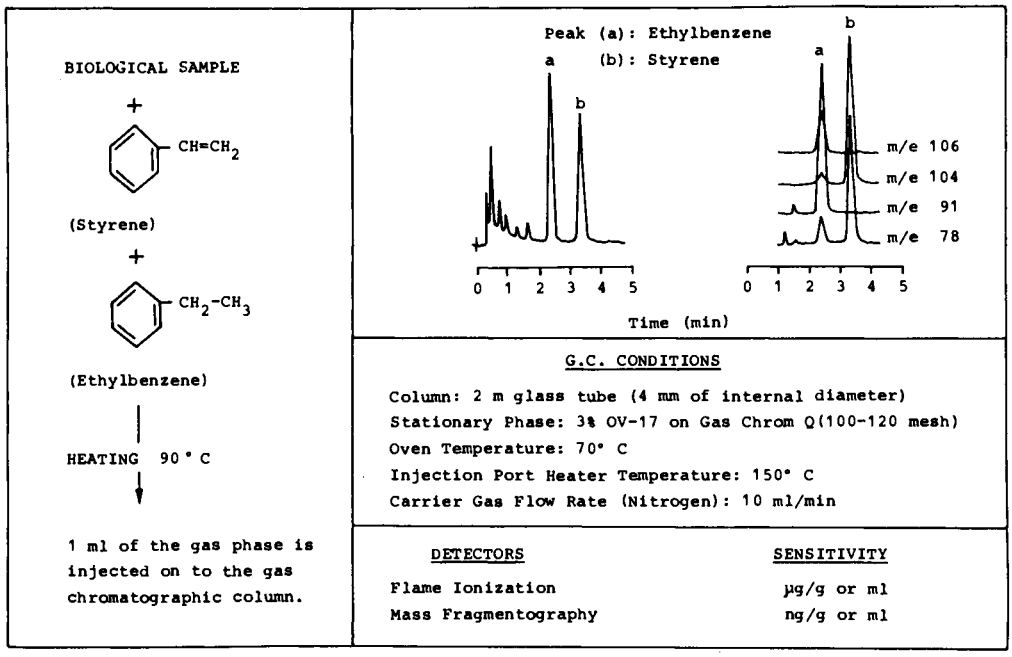

Fig. 4. GLC-MF determination of styrene in biological specimens.

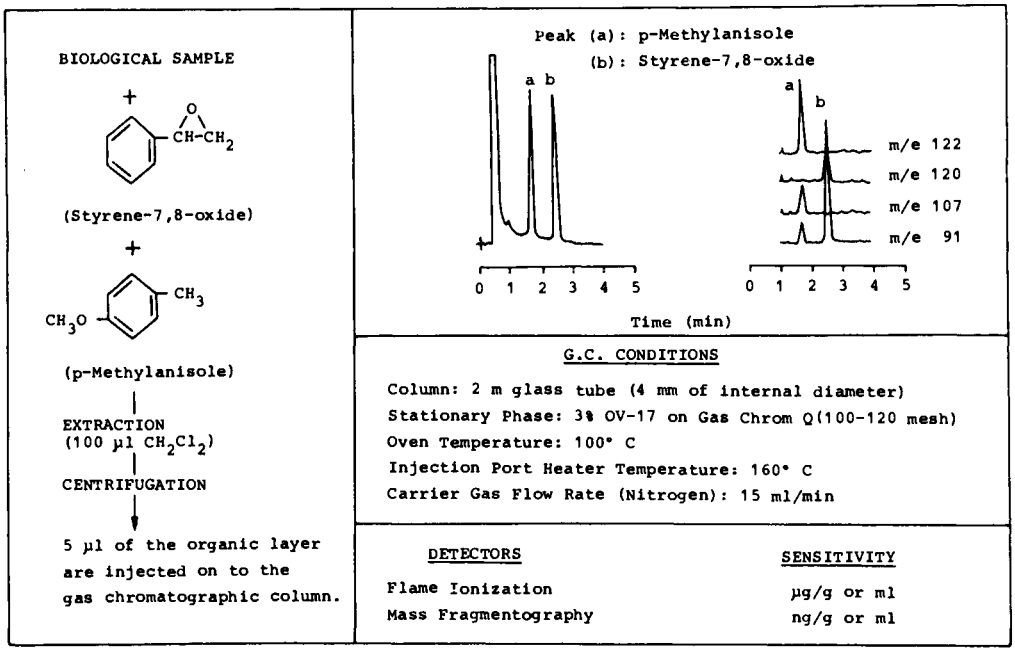

Fig. 5. GLC-MF determination of styrene-7,8-oxide in biological specimens.

GLC-Capillary Column Determination of Benzoic, Mandelic, Phenylglyoxylic and Hippuric Acids

Benzoic acid, mandelic acid, phenylglyoxylic acid and hippuric acid were assayed by GLC-capillary column as described by Pantarotto, Bidoli and Fanelli (1979). Styrene acid metabolites were extracted at acid pH with ethylacetate and converted to their methylesters with dimethylformamide and dimethylacetal as shown in Fig. 6 and 7. Ethylated analogs were used as the internal standards for quantitation.

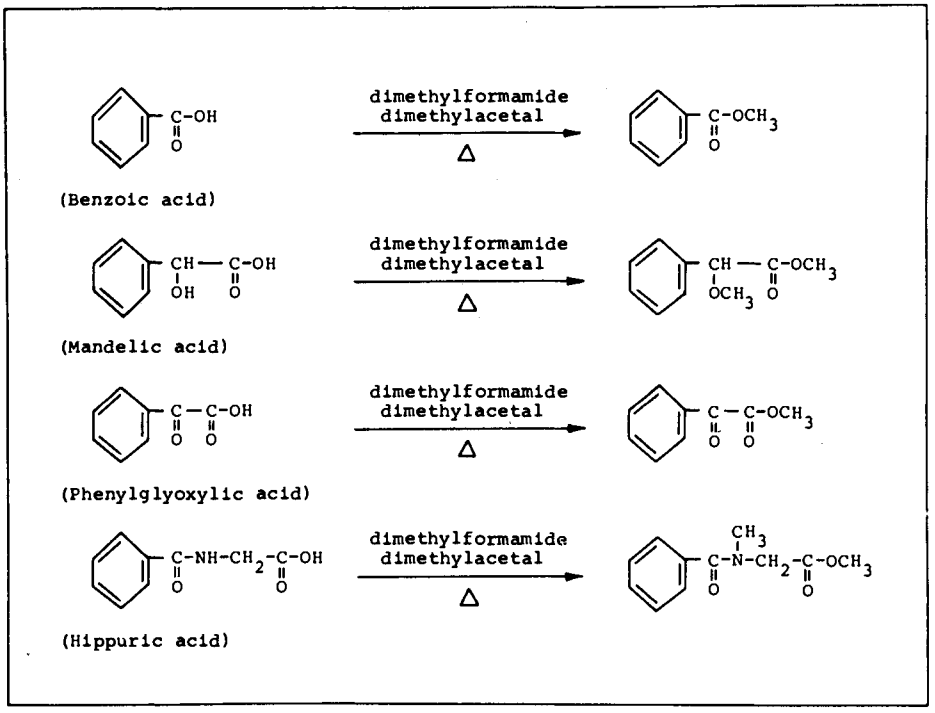

Fig. 6. Permethylation reaction of benzoic acid, mandelic acid, phenylglyoxylic acid and hippuric acid.

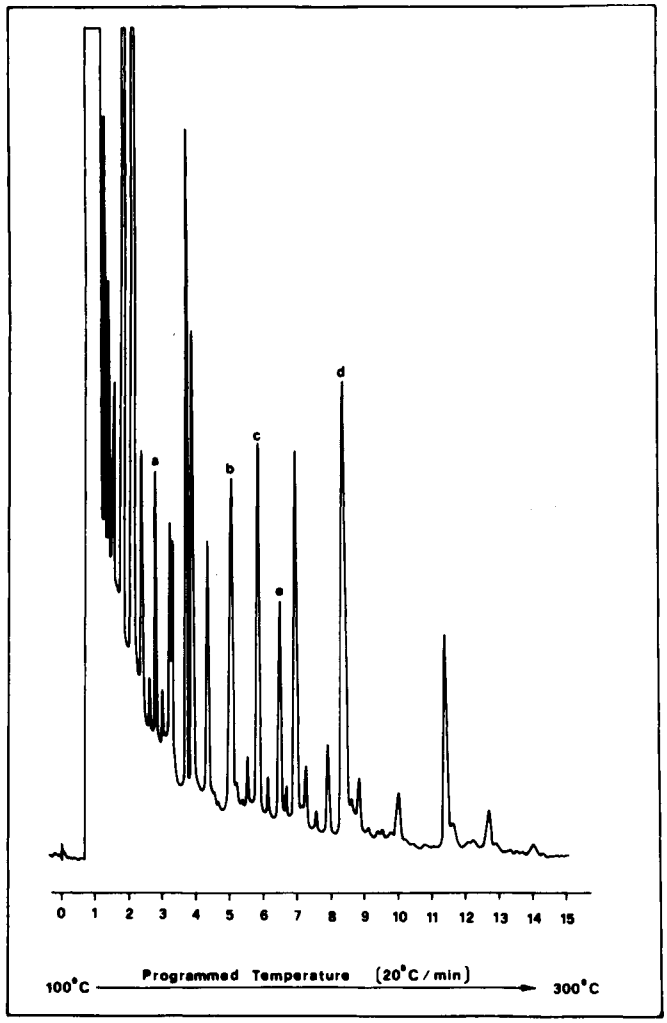

Fig. 7. Capillary column-gas chromatogram of an urine extract from styrene treated mice.
Peak a: benzoic acid methyl derivative.
Peak b: mandelic acid methyl derivative.
Peak c: phenylglyoxylic acid methyl derivative.
Peak d: hippuric acid methyl derivative.
Peak e: mandelic acid ethyl derivative (internal standard).

Mercapturic Acid Metabolites of Styrene

Mercapturic acid metabolites of styrene were assayed according to a photometric method described by Seutter-Berlage and others (1978), essentially a modification of the Ellman procedure (1959).

Reduced Glutathione Levels in Mouse Tissues

Reduced glutathione in the liver, brain, lungs, heart, perirenal fat, spleen and kidneys of control mice was assayed according to a spectrophotometric procedure described by Bernt and Bergmeyer (1974).

Styrene-7,8-oxide Forming Monooxygenase and Styrene-7,8-oxide Hydrase Enzymatic Activities

Styrene-7,8-oxide forming monooxygenase and styrene-7,8-oxide hydrase enzymatic activity in the microsomal fraction of all the tissues examined was assayed according to Belvedere and others (1976) using, respectively, styrene and styrene-7,8-oxide as substrates. A scheme of this procedure is shown in Fig. 8. The method is based on GLC analysis of phenylethylene glycol after its esterification with n-butylboronic acid. The sensitivity depends on the type of detector used (FID or MF).

Glutathione-S-styrene-7,8-oxide Transferase Enzymatic Activity

The activity of the enzyme glutathione-S-styrene-7,8-oxide transferase was determined in the 100,000 g supernatant fraction of the tissues examined according to a radiochemical method described by Hayakawa, Lemahien and Udenfriend (1974) using styrene-7,8-oxide as substrate.

TLC Separation of Styrene Metabolites

TLC was performed on 5 x 20 cm glass plates precoated with silica gel F-254 (Merck) and developed at room temperature in a solvent system consisting of the organic phase from benzene:ethylacetate: acetic acid:methanol:water (14:6:1:3:16). The plates were read under UV light at 254 and 361 nm and checked for radioactivity on a Packard radiochromatogram scanner.

Covalent Binding of ^{14}C-Phenylethylene Glycol to Microsomal Proteins

^{14}C-Phenylethylene glycol covalent binding to microsomal proteins was determined according to Pantarotto and others (1978). The labelled compound (0.5 mM) was incubated at 37°C for 2 h with rat liver microsomal preparations in the presence of an NADPH generating system. Proteins were precipitated by addition of ethanol and then exhaustively extracted with a sequence of solvents in

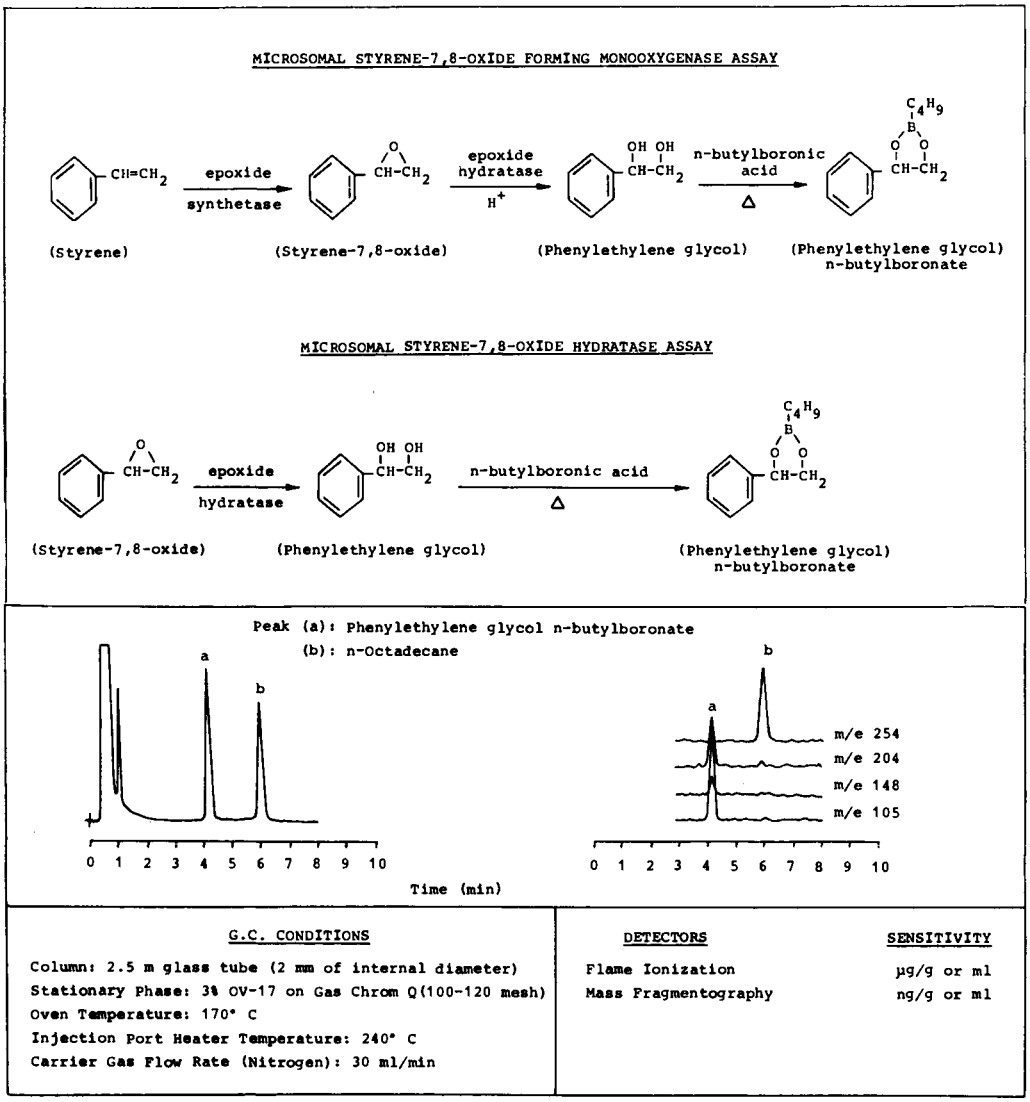

Fig. 8. GLC-MF determination of styrene-7,8-oxide forming monooxygenase and styrene-7,8-oxide hydrase enzymatic activities.

order to remove the remaining ^{14}C-phenylethylene glycol and all the reversibly bound metabolites. The fraction of metabolites covalently bound to microsomal proteins was determined by LSC in a PPO-POPOP mixture after protein solubilization in Soluene^{R}-350 (Packard).

RESULTS AND DISCUSSION

Biochemical Aspects of Styrene Toxicity

Any attempted predictive study of which body districts are most likely to become targets for the toxic effects of a reactive species during metabolism of an exogenous compound, or any attempt to establish why these effects are sometimes selective towards specific organs, can perhaps be approached initially simply by investigating tissue exposure to the metabolic intermediate or product thought to be responsible for known toxicity.

Reports in the literature attribute styrene toxicity to its biotransformation into an epoxide in the 7,8-position (Bardodej, 1978; Härkönen, 1978; Leibman, 1975); this reactive epoxide can be identified and determined quantitatively in liver microsomal preparations where it may be found at relatively high concentrations (Leibman and Ortiz, 1969, 1970).

We therefore first attempted to determine styrene-7,8-oxide *in vivo* in the tissues and organs of animals treated with styrene. However, the *in vivo* half life of a reactive species is often so short that its quantitation becomes impossible. Styrene-7,8-oxide is easily determinable *in vitro* but is hardly even detectable *in vivo* in the different tissues. This is probably because styrene and the 7,8-oxide metabolite are present in low concentrations at the level of both toxifying and detoxifying enzymes in the cell. In our experiments trace amounts of styrene-7,8-oxide were identified *in vivo* in lungs and liver of mice only when they had been pretreated with trichloropropene oxide, an inhibitor of styrene-7,8-oxide hydrase (Oesch and Daly, 1971, 1972; Oesch, Jerina and Daly, 1971; Oesch, 1974). The concentrations, however, were so low that no correlations could be made on the basis of the data obtained.

The problem was overcome by building an experimental model based on four main assumptions: 1) this model only covers forms of toxicity related to formation of a reactive species in the metabolism of styrene, 2) the toxicity of styrene is mainly related to its epoxidation to styrene-7,8-oxide, 3) styrene and styrene-7,8-oxide availability to enzymatic systems and targets in the cell is similar in all the tissues examined, and 4) styrene-7,8-oxide *in vivo* half-life in tissue where it is formed is so short that it cannot migrate to other tissues.

On the basis of the known styrene metabolism and of the above considerations, styrene toxicity in a certain body district may depend on: i) the presence and activity of styrene-7,8-oxide for-

ming monooxygenase, ii) the presence and activity of styrene-7,8-oxide hydrase, iii) the concentration of styrene, substrate of the epoxide forming monooxygenase, and iv) the amount of reduced glutathione and the activity of glutathione-S-styrene-7,8-oxide transferase.

Tables 1 and 2 show the activity of styrene-7,8-oxide forming monooxygenase and styrene-7,8-oxide hydrase in microsomal fractions from the liver and other tissues of male rats, mice, guinea pigs and rabbits, species commonly used in the laboratory. Both enzymes were found in liver, lungs, heart, spleen and kidneys but not in brain or perirenal fat. The liver is the organ with the highest monooxygenase and epoxide hydratase level and is therefore the most important site of styrene activation and styrene-7,8-oxide detoxification. Particularly high styrene-7,8-oxide forming monooxygenase levels were found in the lungs and kidneys of mice and rabbits, monooxygenase activity being even higher in the rabbit lung than the liver.

TABLE 1 Styrene-7,8-oxide Forming Monooxygenase Activity in the Liver and Other Tissues of Male Animals

SPECIES	Styrene-7,8-oxide forming monooxygenase activity (n mol/min/mg protein) ± S.D.						
	Liver	Brain	Lungs	Heart	Perirenal fat	Spleen	Kidneys
Rat	1.95 ±0.27	n.d.	0.34 ±0.08	0.28 ±0.09	n.d.	0.29 ±0.09	0.34 ±0.07
Mouse	3.81 ±0.77	n.d.	2.97 ±0.94	0.38 ±0.01	n.d.	0.48 ±0.06	0.72 ±0.20
Guinea pig	5.20 ±0.90	n.d.	2.55 ±0.10	0.24 ±0.08	n.d.	0.42 ±0.06	0.76 ±0.07
Rabbit	3.82 ±0.36	n.d.	6.67 ±0.85	1.01 ±0.09	n.d.	2.48 ±0.75	2.03 ±0.23

n.d. : not detectable.

Table 3 reports the ratios of microsomal epoxide hydrase to monooxygenase activity, which may be taken as approximate indicators of the balance between deactivating and activating capacity of the various organs. In view of the high ratios of hydrase to monooxygenase activity found in the rat, this species, possibly the one used most widely in drug metabolism studies, can be considered relatively resistant to styrene toxicity. Mice and rabbits seem to have the least ability to inactivate styrene-7,8-oxide. These observations correlate well with the data of Vainio and Mäkinen

TABLE 2 Styrene-7,8-oxide Hydrase Activity in the Liver and Other Tissues of Male Animals

SPECIES	Styrene-7,8-oxide hydrase activity (n mol/min/mg protein) ± S.D.						
	Liver	Brain	Lungs	Heart	Perirenal fat	Spleen	Kidneys
Rat	8.66 ±1.64	n.d.	1.63 ±0.16	2.42 ±0.42	n.d.	1.39 ±0.18	3.11 ±0.31
Mouse	5.91 ±2.60	n.d.	0.83 ±0.23	1.04 ±0.50	n.d.	0.78 ±0.40	2.08 ±0.25
Guinea pig	8.42 ±1.50	n.d.	3.69 ±0.33	4.30 ±0.99	n.d.	3.85 ±1.04	3.95 ±0.62
Rabbit	9.24 ±1.21	n.d.	1.64 ±0.52	0.59 ±0.04	n.d.	2.27 ±0.76	3.40 ±0.20

n.d. : not detectable.

TABLE 3 Ratios of Microsomal Styrene-7,8-oxide Hydrase to Styrene Monooxygenase Activity in Male Animals

SPECIES	Styrene-7,8-oxide hydrase: Styrene-7,8-oxide forming monooxygenase activity						
	Liver	Brain	Lungs	Heart	Perirenal fat	Spleen	Kidneys
Rat	4.44	n.d.	4.79	8.64	n.d.	4.79	9.14
Mouse	1.55	n.d.	0.28	2.73	n.d.	1.62	2.88
Guinea pig	1.61	n.d.	1.44	17.91	n.d.	9.16	5.19
Rabbit	2.42	n.d.	0.24	0.58	n.d.	0.91	1.67

n.d. : not detectable.

(1977) who, on the basis of depression of hepatic non-protein sulphydryl content, defined the mouse as the most vulnerable and the rat as the most resistant species to styrene toxic effects.

The greater affinity of styrene-7,8-oxide forming monooxygenase, compared to styrene-7,8-oxide hydrase, and the considerable inter species variability, seem important factors in establishing which is the most suitable species as a model for toxicity studies. According to these data, the mouse and the rabbit have the highest rates of epoxide formation and the slowest epoxide hydration. This is why we used the mouse in our model for investigating the biochemical mechanisms of styrene toxicity.

Figure 9 shows styrene blood levels in control mice and in mice pretreated with either phenobarbital or 3-methylcholanthrene. The kinetic parameters calculated for these curves are reported in Table 4. Peak styrene blood levels in the pretreated animals were the same as in the control mice. However, significant differences were observed in the areas under the curve (AUC)($p<0.01$) and in the rate constant of elimination (Kel)($p<0.01$), indicating significant induction following phenobarbital pretreatment. These in vivo data correlated well with our previous in vitro induction findings on styrene-7,8-oxide forming monooxygenase and styrene-7,8-oxide hydrase activities (Salmona and others, 1976) and with the Japanese group's data (Ohtsuji and Ikeda, 1971).

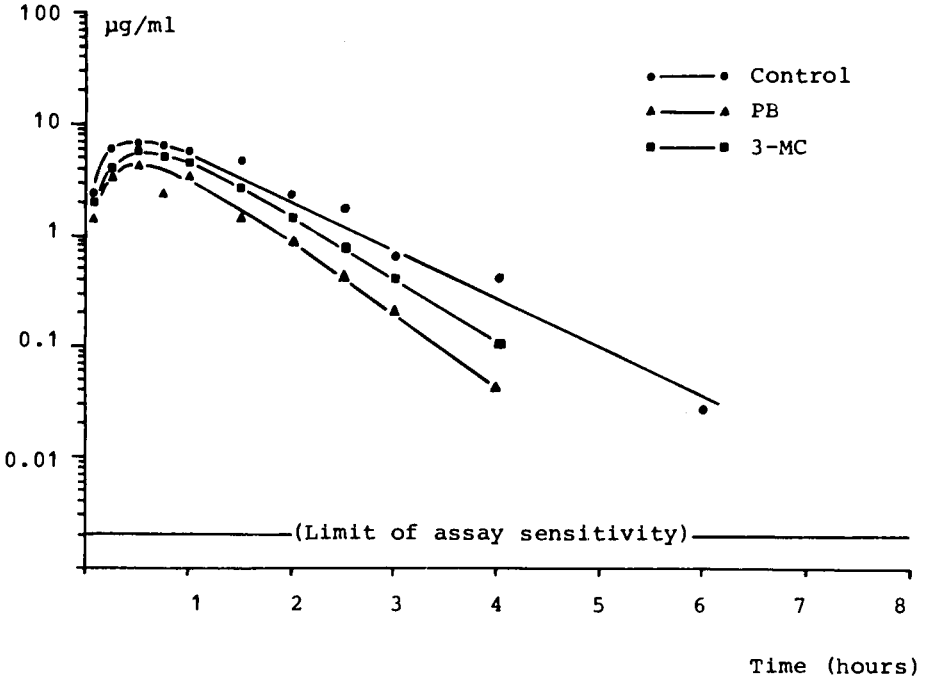

Fig. 9. Styrene kinetics in mouse blood.
PB: phenobarbital.
3-MC: 3-methylcholanthrene.

TABLE 4 Styrene Kinetics in blood of Control Mice and Mice Pretreated with Phenobarbital or 3-Methylcholanthrene

TREATMENT	Blood Peak Level (μg/ml)	AUC (μg/ml x min)	$T_{\frac{1}{2}}$ (min)	Ka (min^{-1})	Kel (min^{-1})	C_0 (μg/ml)	Vd (l/kg)
Control	9.88 ± 1.66	687.65 ± 40.33	41 ± 2	0.056 ± 0.007	0.017 ± 0.001	16.55 ± 1.19	17.27 ± 1.74
Phenobarbital	7.18 ± 0.79	373.00 ± 22.01	24 ± 3	0.043 ± 0.011	0.026 ± 0.002	25.55 ± 1.78	20.37 ± 2.62
3-Methyl-cholanthrene	6.89 ± 0.82	593.54 ± 55.12	31 ± 4	0.056 ± 0.003	0.022 ± 0.001	21.18 ± 2.00	15.47 ± 2.14

Each figure is the mean ± S.D. of at least ten determinations.

TABLE 5 Styrene Distribution in Mice

TISSUE	Tissue Peak Level (μg/g)	AUC (μg/g x min)	$T_{\frac{1}{2}}$ (min)	Ke (min^{-1})	Kel (min^{-1})
Brain	10.74 ± 0.71	515.11 ± 65.07	38 ± 2	0.097 ± 0.037	0.017 ± 0.003
Heart	18.96 ± 2.50	795.00 ± 54.07	43 ± 4	0.082 ± 0.013	0.016 ± 0.001
Lungs	11.73 ± 2.73	887.34 ± 39.19	43 ± 1	0.193 ± 0.024	0.016 ± 0.001
Liver	34.29 ± 3.51	1517.51 ± 143.77	35 ± 1	0.385 ± 0.050	0.019 ± 0.001
Kidneys	28.81 ± 6.72	1741.57 ± 102.24	36 ± 3	0.131 ± 0.019	0.019 ± 0.001
Spleen	40.51 ± 5.82	1479.16 ± 56.89	36 ± 2	0.291 ± 0.061	0.018 ± 0.001
Perirenal fat	242.62 ± 62.79	27557.86 ± 1649.40	40 ± 4	0.039 ± 0.004	0.017 ± 0.003

Each figure is the mean ± S.D. of at least ten determinations.

Table 5 shows styrene distribution in mouse tissues. The compound is rapidly absorbed and distributes in all the tissues examined. Very high area under the curve values were observed in perirenal fat mainly because of styrene's lipid-solubility, as also reported by Withey (1978) in the rat. Absorption and elimination of styrene showed first order kinetics in blood and tissues and, interestingly, the apparent rate constant of elimination from blood ($0.0170\ min^{-1}$) was not significantly different from that from tissues (average $0.0178\ min^{-1}$) indicating a direct equilibrium of tissues and the blood compartment.

Table 6 reports the levels of reduced glutathione.

TABLE 6 Reduced Glutathione Levels in Mouse Tissues

TISSUE	μ mol/g ± S.E.
Brain	0.628 ± 0.008
Liver	3.877 ± 0.121
Lungs	0.300 ± 0.043
Kidneys	0.639 ± 0.064
Heart	0.731 ± 0.044
Spleen	1.952 ± 0.320
Perirenal fat	n.d.

n.d. : not detectable

The highest glutathione concentrations were found in the liver and the lowest in the lungs. Except for the spleen which had almost 2 micromoles per gram of tissue, levels in other tissues examined were similar.

Table 7 shows the activity of glutathione-S-styrene-7,8-oxide transferase in tissues. Again the liver is the major site of glutathione conjugation but activity was high in lungs and kidneys too. It should be recalled here that to make this experimental model for styrene toxicity reliable, all enzymatic activities were determined using homogeneous substrates (i.e. styrene or styrene-7,8-oxide).

Urinary elimination of styrene metabolites by mice is shown in Table 8. The values reported, expressed as % of the dose, indicate that the main detoxification pathway proceeds through styrene-7,8-oxide hydration. Elimination in the urine of phenylethylene

TABLE 7 Glutathione-S-styrene-7,8-oxide Transferase Activity in Mouse Tissues

TISSUE	n mol conjugate/min/mg protein ± S.E.
Brain	1.5 ± 0.7
Liver	26.1 ± 0.3
Lungs	6.5 ± 0.7
Kidneys	5.4 ± 1.8
Heart	2.6 ± 1.2
Spleen	3.4 ± 1.1
Perirenal fat	n.d.

n.d. : not detectable

TABLE 8 Urinary Elimination of Styrene Metabolites by Mice

METABOLITE	% of the dose ± S.E.
Phenylethylene glycol	1.1 ± 0.3
Mandelic acid	31.0 ± 6.1
Phenylglyoxylic acid	13.3 ± 3.7
Benzoic acid	1.5 ± 0.5
Hippuric acid	20.6 ± 4.3
Total mercapturic acids	7.5 ± 2.9

glycol and other metabolites (in both the free and conjugate form) deriving from successive phenylethylene glycol oxidations amounts to about 70% of the dose, the total mercapturic acids fraction (mainly resulting from styrene-7,8-oxide conjugation with glutathione) accounting only for 8%.

On the basis of previous considerations and results, we can integrate the biochemical approach with the in vivo distribution and metabolism findings. A quantitative picture was obtained in terms of tissue exposure to styrene, expressed by the area under the curve, and in the ratio of toxifying to detoxifying activities (styrene-7,8-oxide hydrase and both chemical and enzymatic styrene-7,8-oxide glutathione conjugation) for their respective contributions to total epoxidation of styrene and to its elimination as mercapturic acids or as phenylethylene glycol, mandelic acid, phenylglyoxylic acid, benzoic acid and hippuric acid. The results of this analysis indicate that the lungs, liver and spleen, in that order, are where styrene is most likely to prove toxic.

How these findings are related to styrene toxicity for discrete body tissues in laboratory animals or man can be established through an analysis of the literature. Styrene was shown by Parkki and coworkers (1977) to be hepatotoxic in the rat, an observation which agrees with the same group's findings on in vivo styrene covalent binding to the liver and with previous reports on styrene as the cause of an increase in liver weight (Wolf and others, 1956; Marniemi and others, 1977). Styrene exposure has also been associated by Samedov, Mamedov and Bekeshev (1974) with a decrease in albumin levels in rabbit serum. Recently the International Agency for Research on Cancer in Lyon, reporting on the effects of long-term (100 weeks) oral administration of styrene to mice and rats, stated that, when it was administered weekly at high dose levels (1.350 mg/kg) to 0_{20} mice, styrene significantly shortened their life-span causing severe lesions of the liver, in particular centrilobular necrosis, and an increased incidence and earlier appearance of lung tumours (Ponomarkov and Tomatis, 1978). These results constitute the only weak evidence for the carcinogenicity of styrene when administered at high concentrations to animals.

The most noteworthy findings, reported in the 1960's, on tissue injury by styrene in exposed workers are: hepatomegaly, toxic hepatitis and splenomegaly (Bardodej and others, 1960; Ermolova and others, 1965; Katz, 1962). Lymphocytosis, lowered serum albumin levels and urinary secretion of coproporphyrin have also been reported(Klein and Zak, 1969; Lukoshkina and Alekperov, 1973; Orlova and Solovéva, 1962). Studies made in 1978 in the United States by the National Institute of Occupational Safety and Health, the University of North Carolina and the Department of Community Medicine of the Mount Sinai School of Medicine in New York showed evidence of a risk of death due to haemopoietic and lymphatic malignancies among workers producing synthetic rubbers (Meinhardt, Young and Hartle, 1978; Nicholson, Selikoff and Seidman, 1978).

Neurophysiological disturbances in exposed animals and humans have been also described (Gamberale and Hultengren, 1974; Lilis and others, 1978; Savolainen and Pfäffli, 1977, 1978; Stewart and others, 1968); studies with labelled styrene and styrene-7,8-oxide revealed the possibility of covalent binding between these substrates and macromolecules of the nervous system (Savolainen and Vainio, 1977). At present we cannot find any correlation between central nervous system toxicity and our biochemical findings in brain mainly because in our studies the brain was always

considered in toto and not as an ensemble of discrete regions.

Metabolism Studies

New perspectives on the toxicity of styrene are offered by the identification of phenolic metabolites during styrene biotransformation. This new metabolic pathway, even though ancillary, appears of interest since phenolic compounds may be formed as a result of chemical rearrangements of unstable arene oxides, reactive species which might be implicated in styrene toxicity, or by dehydration of dihydrodiol metabolites as shown in Fig. 10.

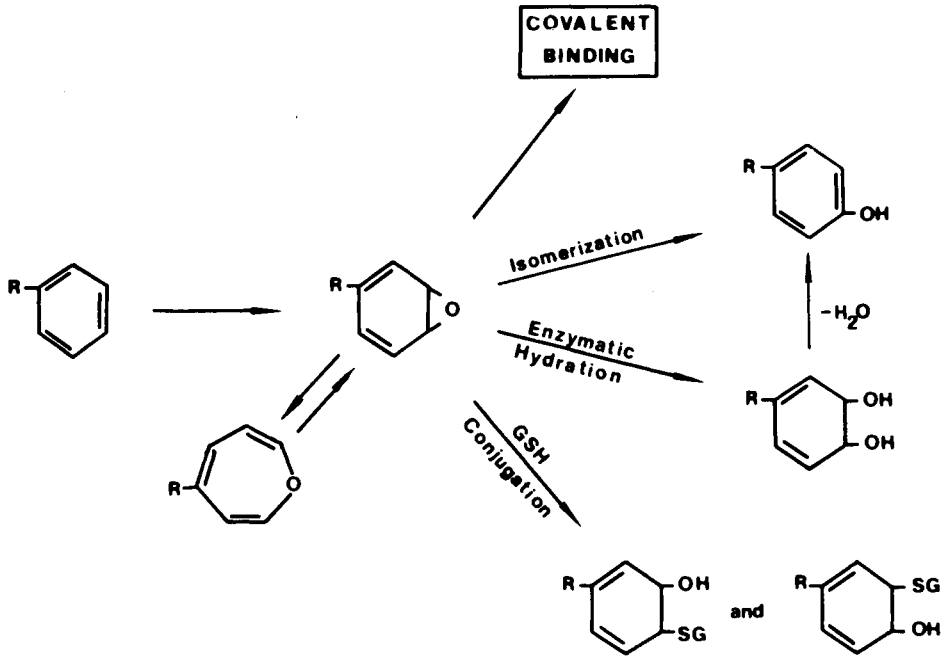

Fig. 10. General metabolism of arene oxides

Styrene can be first metabolized into an unstable arene oxide in the 3,4-position, in equilibrium with an oxepin form, which subsequently indergoes spontaneous rearrangement to form 4-vinylphenol.

Our interest in arene oxides as both metabolic intermediates and mediators of toxic effects led us to investigate styrene biotransformation in rats with particular reference to the formation of phenolic metabolites.

Table 9 shows the Rf values of some known styrene metabolites and three other biotransformation products, metabolite I, II and III, isolated from the ethylacetate urine extracts of animals given

unlabelled and ^{14}C-styrene.

TABLE 10 Rf Values on Thin Layer Chromatography of Styrene Reference Compounds and Metabolites Isolated from Rat Urine

COMPOUND	Rf Value
Phenylethylene glycol	0.45
Mandelic acid	0.06
Phenylglyoxylic acid	0.12
Benzoic acid	0.59
Hippuric acid	0.22
Metabolite I	0.18
Metabolite II	0.52
Metabolite III	0.04

Solvent system: organic phase from benzene:ethylacetate: acetic acid:methanol:water (14:6:1:3:16).

Figure 11 shows a gas chromatogram obtained by co-injecting with trimethylanilinium hydroxide the ethylacetate extract from urine of rats given unlabelled styrene. Analysis was made at a programmed temperature from 100 to 300°C. In the chromatogram, exception for peak 3 which is due to N,N-dimethylaniline from trimethylanilinium hydroxide, the peaks with arabic numbers correspond to known styrene metabolites. The mass spectrum corresponding to peak 4 is shown in Fig. 12. The molecular ion and base peak at m/e 134 and the presence of the other fragments at m/e 119, 108, 91 (vinylcyclopentadienyl cation), 77 and 65 (cyclopentadienyl cation) are all characteristic of the vinylphenol methyl derivative structure. We therefore assigned this metabolite the structure of vinylphenol, probably hydroxylated in the 4-position. Peaks 5, 6, 7 and 8 are the methyl derivatives of benzoic acid, mandelic acid, phenylglyoxylic acid and hippuric acid respectively. Peaks 1 and 2, identified as 1- and 2-methoxy-1-phenylethylene, were degradation products of phenylethylene glycol as shown in Fig. 13. This degradation is a loss of water probably catalyzed by the slight acidity of the stationary phase (3% OV 17) of the gas chromatographic column at high temperature. The two alcohols formed in the presence of trimethylanilinium hydroxide are then readily converted to the corresponding methylethers.

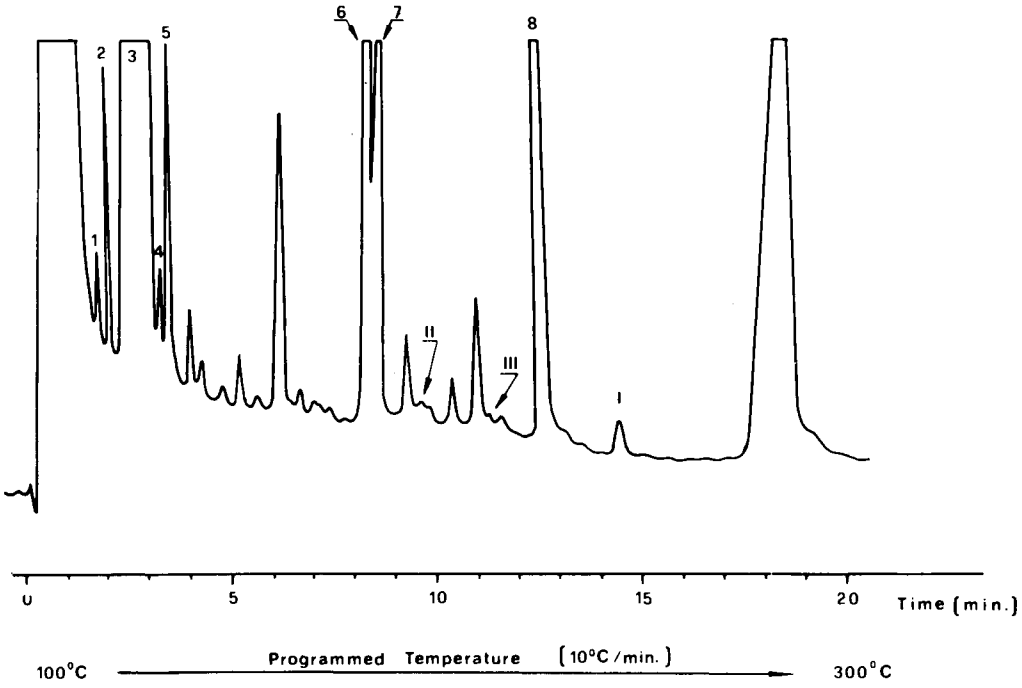

Fig. 11. Gas chromatogram of the ethylacetate extract from the urine of styrene-treated rats. The analysis was performed at a programmed temperature of 100-300°C, the ethylacetate extract being co-injected with trimethylanilinium hydroxide.

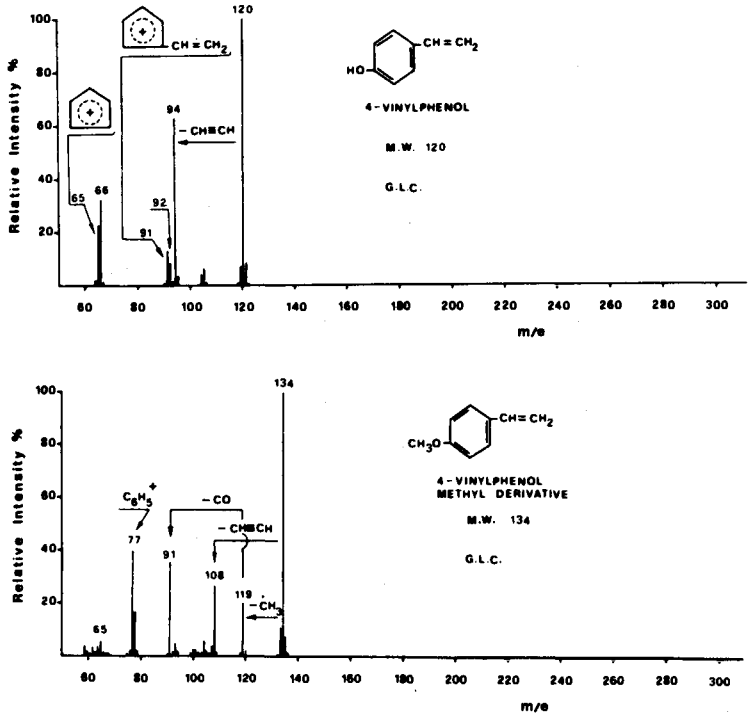

Fig. 12. Mass spectra of 4-vinylphenol and its methylated derivative.

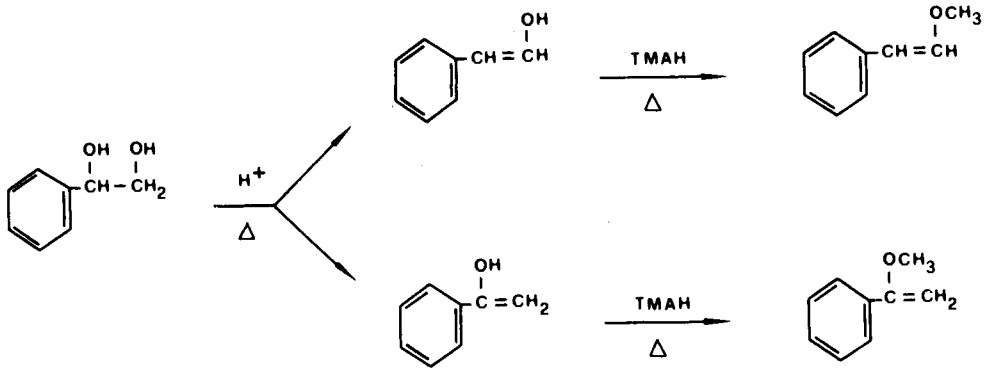

Fig. 13. Gas chromatographic degradation of phenylethylene glycol to 7- and 8-hydroxystyrene.

In Fig. 11, peaks with roman numbers are unidentified metabolites.

Figure 14 shows the mass spectra for metabolite I either through direct introduction (DIS) of the eluate of the spot at Rf 0.18 into the ion source of the mass spectrometer (upper spectrum) or by GLC after on column permethylation (lower spectrum). The simple and clear fragmentation and the presence of the cyclopentadienyl cation at m/e 65 were consistent with the structure of a hydroxy derivative of hippuric acid. Metabolite I was therefore identified as hydroxyhippuric acid, probably at the para position.

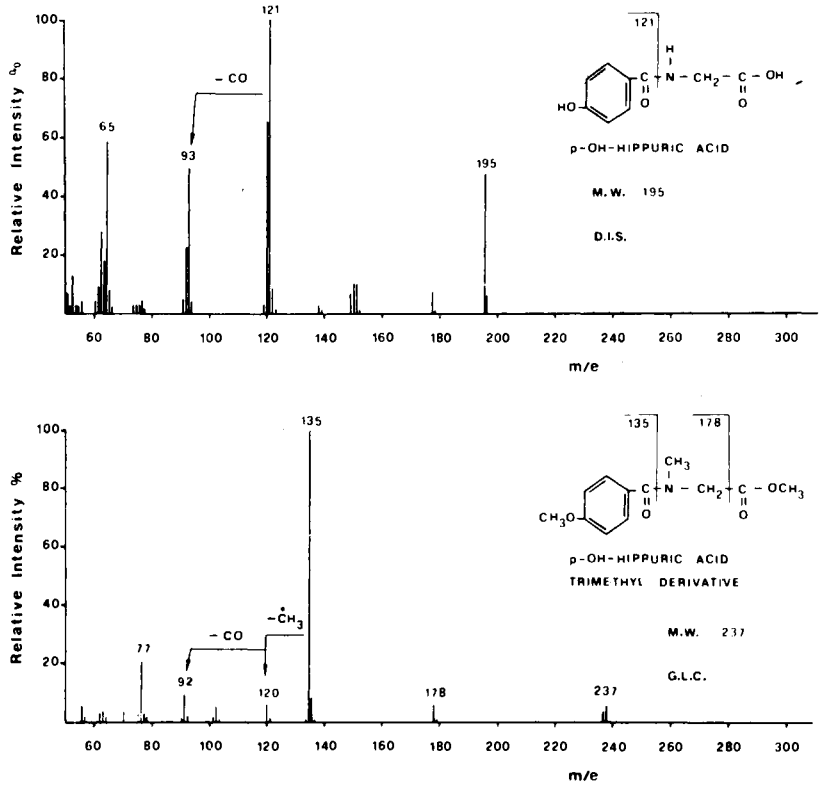

Fig. 14. Mass spectra of p-hydroxyhippuric acid obtained by the direct inlet system and by gas-liquid chromatography after permethylation.

The presence of this hydroxylated metabolite suggested that hydroxymandelic acid and hydroxybenzoic acid might also be found in the urine. Identification of both these metabolites was simplified by the availability of pure p-hydroxymandelic and p-hydroxybenzoic acids to use as standards.

Figure 15 shows the mass spectra of p-hydroxymandelic acid obtained by DIS (upper spectrum) or by GLC after permethylation (lower spectrum). Fragmentation of these compounds is very simple, the

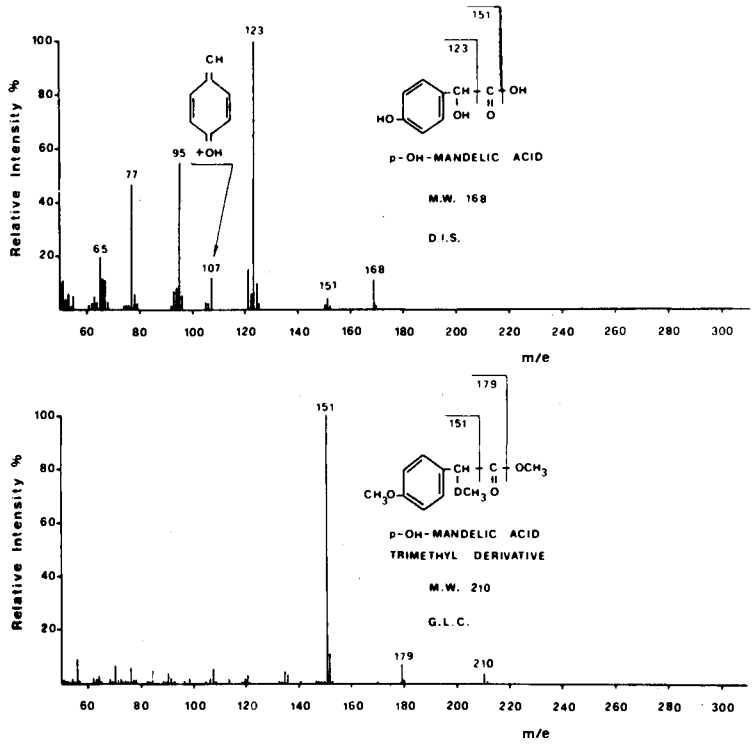

Fig. 15. Mass spectra of p-hydroxymandelic acid obtained by the direct inlet system and by gas-liquid chromatography after permethylation.

ion at m/e 107 being a stable oxonium ion, while the ions at m/e 95 and 77 may arise as shown in Fig. 16. The ion at m/e 123, in equilibrium with a more stable seven membered form, loses carbon monoxide giving rise to the ion at m/e 95, a hydroxy analog of the benzenium ion, which undergoes dehydration to give the ion at m/e 77. Metastable peaks have been observed confirming these transitions.

Figure 17 shows the mass spectra of p-hydroxybenzoic acid obtained by DIS (upper spectrum) or by GLC analysis after derivatization with trimethylanilinium hydroxyde (lower spectrum). These spectra are again very simple and characteristic of their respective structures.

On this basis, p-hydroxymandelic acid and p-hydroxybenzoic acid were identified in the urine as their methylated derivatives by means of mass chromatography. The mass spectrometer was focussed on the ions at m/e 166, 135 and 107 characteristic of the dimethyl derivative of p-hydroxybenzoic acid and on the ions at m/e 210, 179

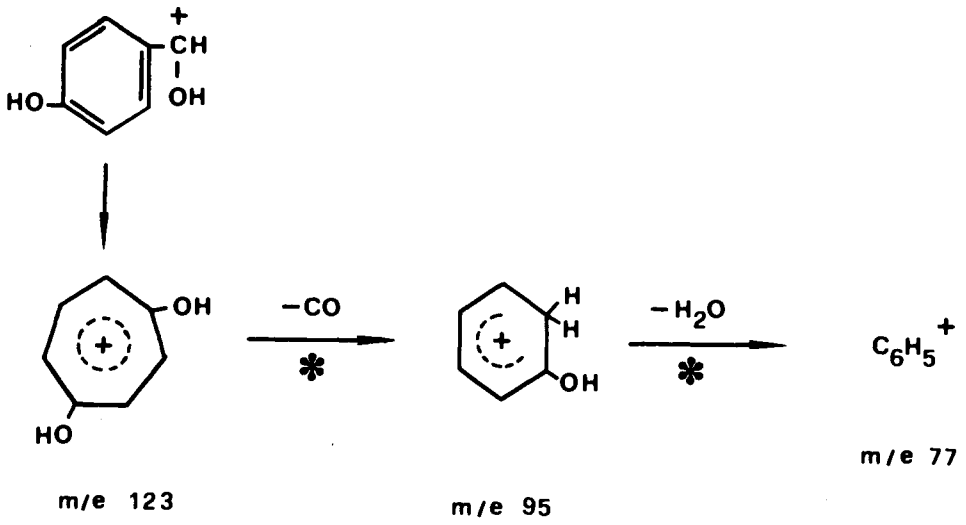

Fig. 16. Rationale for the loss of carbon monoxide and water from the ion at m/e 123.

and 151 characteristic of the spectrum of p-hydroxymandelic acid as its trimethyl derivative. Figure 18 shows the resulting mass chromatogram which clearly indicates the presence of these two phenolic metabolites in urine.

Figure 10 proposes the formation of phenolic metabolites as a result of isomerizations of unstable arene oxides, reactive intermediates which can bind covalently to cellular macromolecules, conjugate with glutathione or be hydrated to dihydrodiols.

An indirect demonstration of the intermediacy of arene oxides in the formation of styrene phenolic derivatives is given by our studies on the covalent binding of ^{14}C-phenylethylene glycol to microsomal proteins. The labelled phenylethylene glycol enabled us to distinguish between covalent binding resulting from epoxidation at the 7,8-double bond position from binding due to oxidation of the aromatic ring at the 3,4-position. Table 11 gives the results of the covalent binding studies. The process NADPH dependent, is significantly induced by pretreatment with 3-methylcholanthrene, while no changes in binding were observed in microsomal preparations obtained from the liver of phenobarbital pretreated rats. Binding was greatly inhibited when glutathione was added to the incubation mixture at a concentration of 1 mM.

These data indicate that aromatic hydroxylation of styrene is catalyzed by a cytochrome P-448 dependent monooxygenase. Together with the studies of Watabe and coworkers (1978b) on the comparative mutagenicity of styrene, styrene-7,8-oxide and styrene-3,4-oxide toward Salmonella typhimurium TA 100, these findings suggest that this new metabolic pathway leading to the formation of phe-

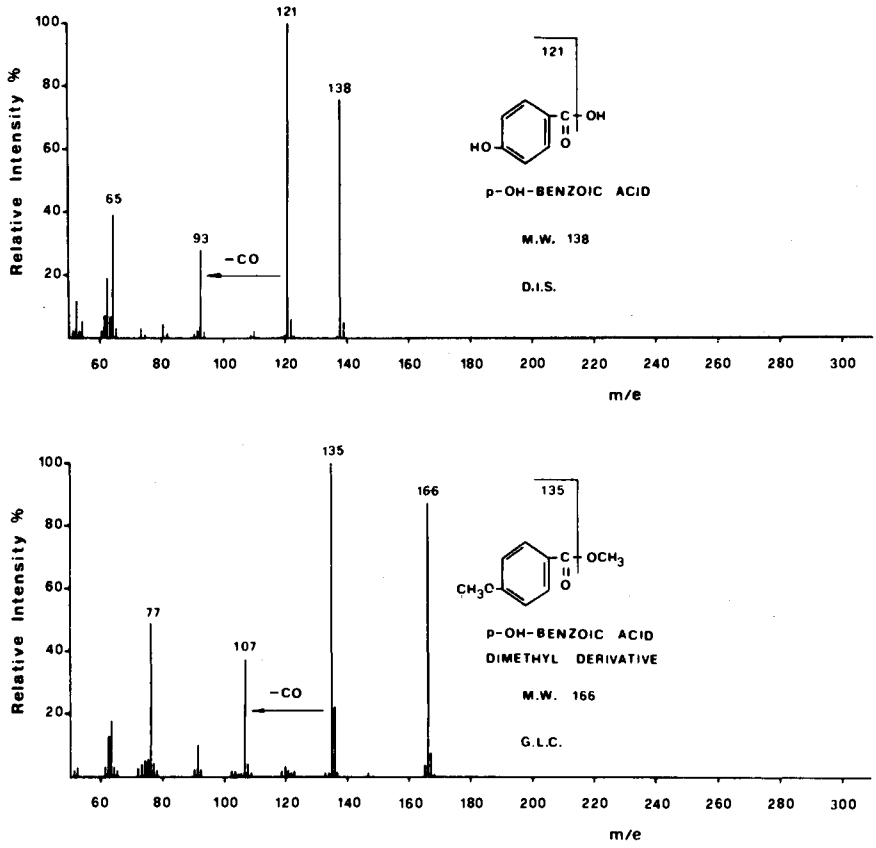

Fig. 17. Mass spectra of p-hydroxybenzoic acid obtained by the direct inlet system and by gas-liquid chromatography after permethylation.

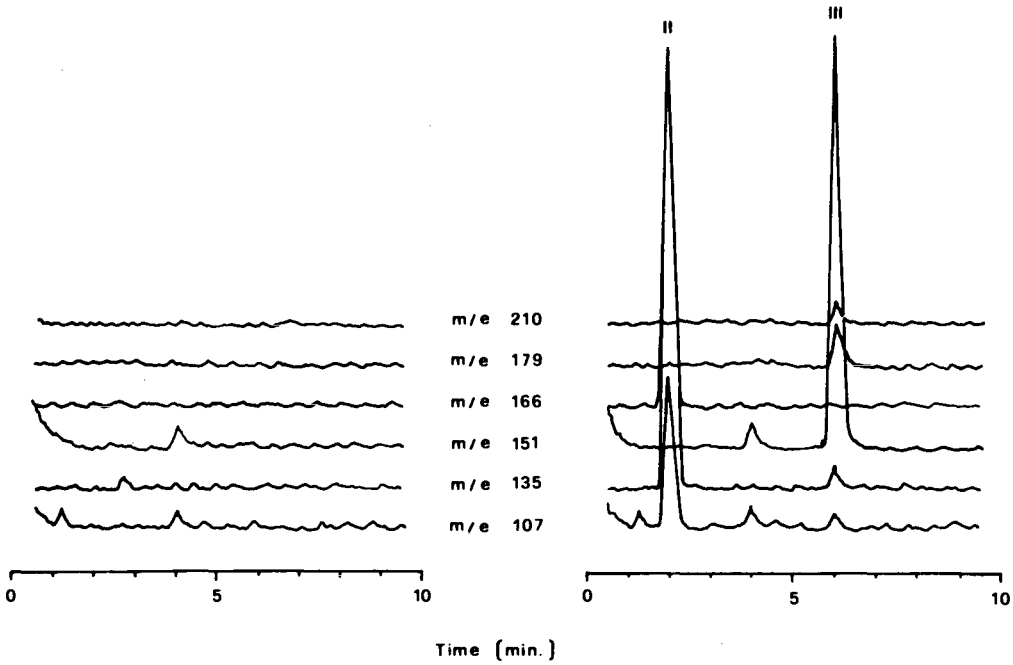

Fig. 18. Mass chromatograms of the ethylacetate extracts from the urine of controls (on the left) and styrene-treated rats (on the right). The analyses were performed at a column temperature of 170°C, the ethylacetate extracts being co-injected with trimethylanilinium hydroxide.

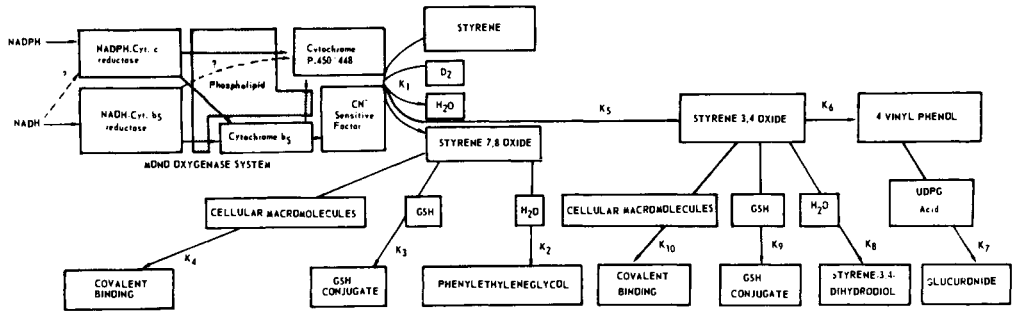

Fig. 19. Biochemical mechanisms of styrene metabolism and toxicity.

TABLE 11 Irreversible Protein Binding of ^{14}C-Phenylethylene Glycol (0.5 mM) Catalyzed by Rat Liver Microsomes in the Presence of an NADPH-Generating System After 120 Min of Incubation

SAMPLE	Phenylethylene glycol metabolites irreversibly bound to 1 mg of microsomal protein (d.p.m.)*	% Radioactivity covalently bound
Boiled control microsomes + NADPH	29 ± 4	0.014
Control microsomes - NADPH	26 ± 1	0.012
Control microsomes + NADPH	73 ± 4	0.034**
Phenobarbital-induced microsomes + NADPH	85 ± 5	0.040
3-Methylcholanthrene-induced microsomes + NADPH	118 ± 9	0.056***
Control microsomes + NADPH + glutathione (1 mM)	41 ± 3	0.019****

*Each value is the mean ± S.E. of four determinations.
** $p<0.01$ compared with boiled control microsomes + NADPH and control microsomes - NADPH.
*** $p<0.01$ compared with control microsomes + NADPH and phenobarbital induced microsomes + NADPH.
**** $p<0.01$ compared with control microsomes + NADPH.

nolic products during styrene biotransformation, even though it occurs to only a limited extent, may play an important role in the toxicity of this compound.

CONCLUSIONS

With this study, using styrene as a model compound, we wanted to focus on two main concepts which must be considered in toxicological studies on environmental chemicals and on exogenous substances in general.

We have shown that the characterization of even minor metabolic pathways which proceed through the formation of highly reactive intermediates may be of importance with a view to better analysis of all the possible causes of any particular type of toxicity, especially when a compound may be activated on different sites of its moiety, giving rise to electrophilic intermediates with different chemical reactivity. It is known in fact that marked toxicity may not necessarily be determined by a quantitatively major metabolism.

The second aspect is our biochemical approach to the study of the mechanisms at the basis of toxicity caused by a chemical in discrete body districts. The results reported show how important it is for this approach to be used as complementary to long-term studies of carcinogenesis and/or toxicity in general.

In order to further these studies, the in vivo covalent binding of styrene to cellular macromolecules (proteins, DNA and RNA) in different tissues of mice under various experimental conditions, and the capacity of nuclear membranes to metabolize this chemical are at present being investigated in our laboratory with the aim of elucidating the finer biochemical mechanisms of certain toxic effects and therefore the predictive value of our toxicity model.

Figure 19 shows the scheme that we will follow in further studies.

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Computerized GC/MS for Solving Problems in Water Pollution

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ABSTRACT

The transport of industrial organic chemicals from their source, into the Delaware River, through various treatment facilities, and into Philadelphia's finished drinking water was studied using water samples collected in August 1977. Solvent extraction, liquid chromatographic clean-up, and gas chromatographic mass spectrometry were used for compound separation and identification. Results confirmed discharge sources for many previously identified compounds. Furthermore, it was shown that many of these compounds circulated into Philadelphia's drinking water, and that the various water and waste treatment facilities had a minimal effect on the organic levels. For all chemicals, dilution processes were responsible for the largest reduction in organic concentrations.

KEYWORDS

gas chromatographic mass spectrometry; drinking water; Delaware River water; industrial wastewater; water treatment effectiveness; solvent extraction; organic water pollutants.

INTRODUCTION

Nearly 100 organic compounds of biologcial, municipal, and industrial origin have been identified in the Delaware River (Sheldon and Hites, 1978). Among the industrial contaminants, several compounds seemed to be coming from a specific plant in the Philadelphia area. Furthermore, relatively high levels of anthropogenic chemicals were observed in the river near the Philadelphia area, indicating that they may be entering the city's drinking water. We have, therefore, traced the movement of various industrial chemicals from their origin, through the river, and into Philadelphia's drinking water. This paper is a report on these studies.

The Sampling Area. Only a small segment of the Delaware River, lying just north of Philadelphia, was studied. A schematic diagram of the complete sampling area is shown in Fig. 1. General flow and hydrolic characteristics of the river have been discussed previously (Sheldon and Hites, 1978). The box in the upper left hand corner of Fig. 1 represents a plant in the Phila-

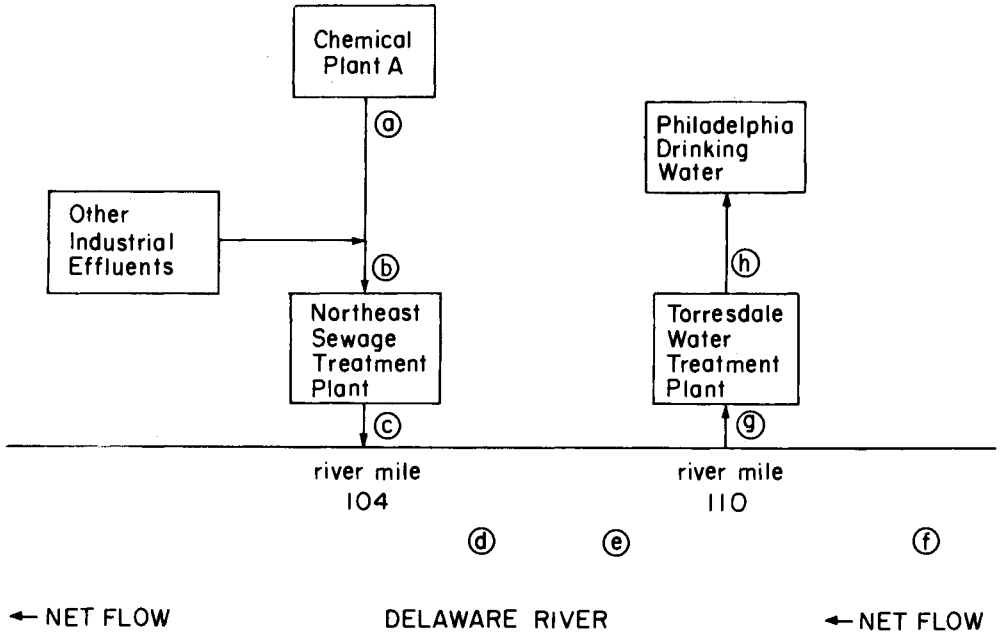


Fig. 1. The sampling area, showing collection sites. River milages are measured upstream from the mouth; net flow procedes from right to left.

Table 1. Sampling Scheme Giving Details of Timing, Sizes, Types, and Locations (see Figure 1).

Location	collection period	size(l)	sampling interval	number of samples	type
a) Plant A effluent	8/23 12PM to 8/24 12PM	0.5	-	1	continuous
b) Northeast influent	8/24 2AM to 8/25 2AM	0.5	4 hr	7	grab
c) Northeast effluent	8/24 8AM to 8/25 8AM	1	4 hr	7	grab
d) River mile 106	8/25 10AM	23	-	1	grab
e) River mile 108	8/25 10:30AM	23	-	1	grab
f) River mile 118	8/25 11AM	23	-	1	grab
g) Torresdale influent	8/25 8AM to 8/26 8AM	4	4 hr	7	grab
h) Torresdale effluent	8/25 8PM to 8/26 8PM	4	4 hr	7	grab

delphia area which we will refer to as Plant A. This plant does not discharge its wastewater directly into the river, but rather it discharges into the city sewer along with several other industrial users. These combined industrial wastes are treated at the City of Philadelphia's Northeast Sewage Treatment plant using classical secondary treatment methods. The treated effluent is then discharged into the Delaware River at river mile 104.

Water flow in this segment of the river is dominated by tidal movement rather than downstream river flow; tidal volumes are an order of magnitude greater than downstream river flows. During periods of normal flow, effluents discharged into the river travel approximately 7 miles upstream during high tide. Under these conditions, water flow in the upstream direction is sufficient to transport chemicals from the sewer outfall upstream to the intake pipes of Philadelphia's Torresdale drinking water facility at river mile 110. Intake valves for this plant are open only during high tide (Radziul, 1977) making industrial waste contamination of the city's drinking water not only possible but probable. Water entering the drinking water plant is treated using standard techniques: prechlorination; settling; coagulation (ferric chloride, alum, and lime); disinfection; flocculation; and filtration (rapid sand filters). After a final chlorination step, drinking water is distributed throughout the city. Water from this treatment facility provides the city of Philadelphia with approximately 50% of its finished drinking water.

EXPERIMENTAL

Samples were collected in late August 1977 from sites a to h as shown in Fig. 1. Our purpose was to follow a 24 hour slug of industrial wastes through the cycle from Plant A to the finished drinking water. The sampling scheme was designed to account for retention times between the various sampling locations, as well as for tidal movement in the river. Details of this sampling regime are outlined in Table 1.

The composite sample from Plant A was taken from a 5 gallon continuous sampler after the 24 hour sampling period. All other samples were composites of individual grab samples collected at a particular location. River water samples were collected approximately 100 yards from the western shore at the designated river mile.

All samples were collected in glass bottles fitted with teflon-lined screw caps. Methylene chloride and hydrochloric acid were added to the water samples at the collection site in order to minimize biological degradation and to start the extraction process. Since waste effluents from Plant A do not support microbial activity, sample preservation in the 24 hour continuous sampler was not needed.

All samples were stored in the dark. Small samples were kept on ice during transport to the laboratory. Larger samples were refrigerated as soon as possible after collection.

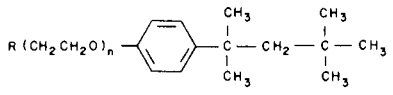
Analytical techniques and instrumentation for the concentration, separation, and identification of sample components have been discussed in detail elsewhere (Sheldon and Hites, 1975). In general, analytical techniques used in this study included solvent extraction, liquid chromatographic fractionation, high resolution gas chromatography, computerized gas chromatographic mass

spectrometry (GC-MS) in both the electron impact (EI) and chemical ionization (CI) mode, and high resolution mass spectrometry (HRMS).

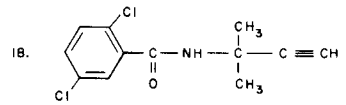
Concentration values are semiquantitative and are based on standard curves for selected compounds. Estimated errors in quantitation are approximately ±20% in Plant A's waste effluent, ±50% in the Northeast influent and effluent and the river water, and an order of magnitude in the finished drinking water.

RESULTS AND DISCUSSION

All of the compounds identified in the industrial wastewater, the municipal sewage effluent, the river water, and Philadelphia's finished drinking water are listed in Table 2. Some structures are given in Fig. 2. Estimated concentrations have been included for most of the abundant compounds. The compounds in Table 2 are listed according to location of first appearance. Within each of these groups, chemicals have been subdivided by compound type. This arrangement allows for both a quick identification of specific pollution sources and for a facile appraisal of the movement of these chemicals in the aquatic system.



7. R = OH, n = 1
8. R = OH, n = 2
9. R = OH, n = 3
10. R = OH, n = 4
11. R = OH, n = 5
12. R = Cl, n = 2
13. R = Cl, n = 3



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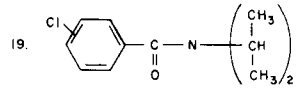

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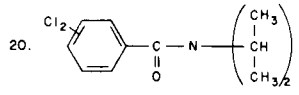

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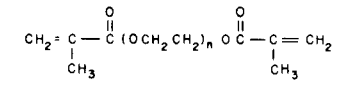

64. n = 2
65. n = 3
66. n = 4

Fig. 2. Structures of selected organic compounds found in the Delaware River (see Table 2).

Mass Spectral Interpretation. The interpretation of the mass spectra of certain compounds proved to be quite interesting, and these cases will be reviewed here. Figure 3 shows the EI mass spectrum of compound 13; the elemental composition of m/e 285 (obtained from HRMS) is included. An electron impact fragmentation pattern of 63, 65, 107, 109, 151, and 153 is characteristic of a mono-chlorinated ion with 44 mass units adducts. Previous identifications of chlorinated ethylene glycols suggested that this should be a similar compound with m/e 63 due to $ClCH_2CH_2$, m/e 107 to $ClCH_2CH_2OCH_2CH_2$, and m/e 151 to $ClCH_2CH_2OCH_2CH_2OCH_2CH_2$. Ions at 77, 91, and 135 are characteristic of C_3-phenolic compounds. A combination of these fragments accounts for the base peak at 285 (see Fig. 3). A mass chromatogram indicated a very weak molecular ion at m/e 356 suggesting that a C_5H_{11} fragment should be added to the 285 ion to give compound 13. The hypothesized structure was synthesized by chlorinating the hydroxy compound (no. 9) with PCl_3. The GC retention time and mass spectrum for the unknown compound were identical to the synthetic compound. Compound 12 was similarly identified. We should point out that compounds 12 and 13 are not artifacts formed by the chlorination of compounds 8 and 9 during the course of sample analysis.

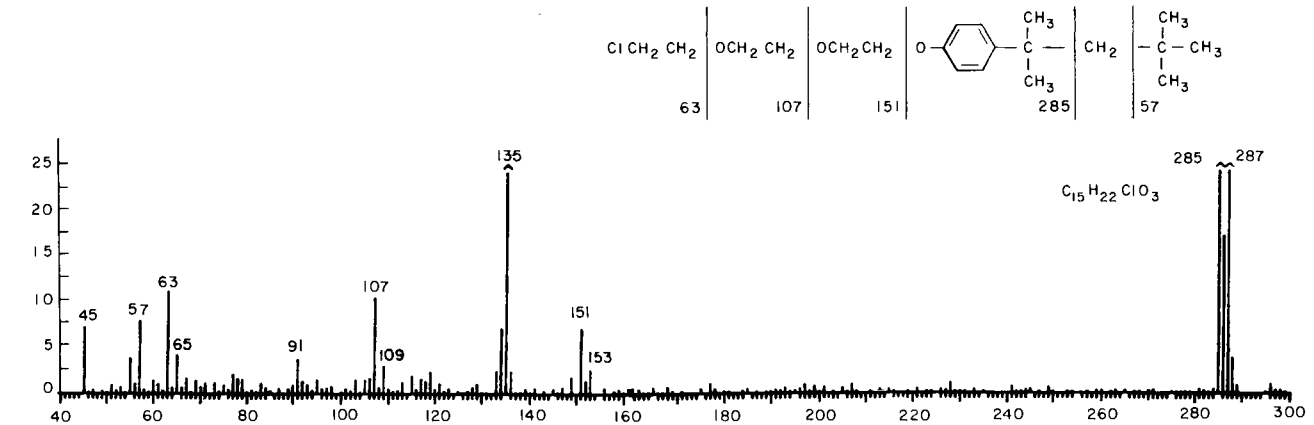

Fig. 3. Electron impact (70 ev) mass spectrum of 1-chloro-2-(2-[2-(p-1',1',3',3'-tetramethylbutylphenoxy)ethoxy]ethoxy)ethane, compound 13. The relative intensities have been expanded by a factor of 4; the intensities of the off-scale peaks are 135 (34%), 285 (100%), and 287 (37%).

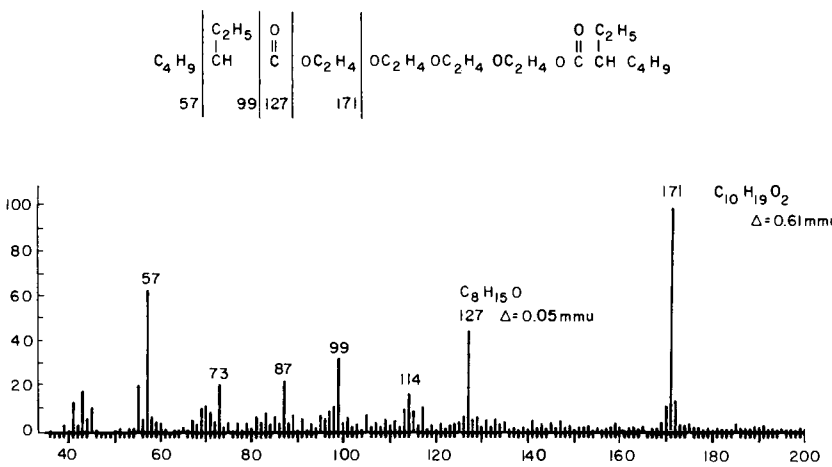

Fig. 4. Electron impact (70 ev) mass spectrum of tetraethyleneglycol-di(2-ethylhexanoate), compound 71. The elemental compositions of m/e 127 and 171 were established by high resolution mass spectrometry; the Δ values indicate the error (in milli-mass units) between measured and calculated exact masses.

Since these two groups of compounds were separated during LC fractionation prior to GC analysis, there was no opportunity for their interconversion.

The plasticizer, tetraethyleneglycol-di(2-ethylhexanoate) (compound 71) was interesting because it was the most challenging to identify. The electron impact mass spectrum for this compound (Fig. 4) shows an intense ion at m/e 171 and less abundant ions at m/e 127, 99, 87, and 57. High resolution mass spectrometry established the elemental compositions of m/e 171 and 127 (see Fig. 4). As we have seen, the small fragment ion at m/e 45 and the large neutral loss of 44 mass units (171 to 127) are characteristic of ethylene glycol compounds. GC retention time suggested a rather high molecular weight despite the absence of any high mass fragments in the EI mode. Methane CI gave no additional information on molecular weight, but isobutane CI showed an M+1 ion at 447. An elemental composition for the molecular ion of $C_{23}H_{46}O_7$ was hypothesized based on the rather saturated composition of m/e 171. Gas chromatography using a nitrogen-phosphorus flame detector did not contradict this hypotheses. A search of the EPA TSCA list and Chemical Abstracts for industrial compounds corresponding to this molecular composition indicated that tetraethyleneglycol-di(2-ethylhexanoate) (a plasticizer patented and produced by one of the companies along the river) was a possibility. Identification and approximated concentrations of the compound were verified using the authentic commercial product. The triethyleneglycol homolog (compound 70) was identified in a similar manner.

Identification of Contamination Sources. For an overview of the occurence and environmental significance of many of the compounds listed in Table 2, the reader is referred to our previous paper on the Delaware River (Sheldon and Hites, 1978). During the following discussion only those compounds which were not previously identified in the Delaware River or which gave some insight into the movement of chemicals through the various treatment processes and in the Delaware River will be considered.

The first objective of this study was to verify that Plant A was the specific source for a set of previously identified compounds. These compounds included 1,2-bis(chloroethoxy)ethane (compound 6), the phenyl glycols (compounds 7-11), the chlorinated phenyl glycols (compounds 12 and 13), DDE (compound 17), dichlorobenzophenone (compound 16), and the binaphthylsulfones (compound 37).

Our data (see Table 2) verify that these chemicals are, in fact, being discharged from Plant A along with various other phenolic (compounds 1-5), chlorinated (compounds 18-20), and esterified species (compounds 25-26). All of the above compounds are either commercial products manufactured at Plant A or are process by-products.

The commercial herbicide, 2,5-dichloro-N(1,1-dimethyl-2-propynl)benzamide (compound 18) was discharged in Plant A's waste effluent in relatively high concentrations (500 ppb). We should point out that this compound was not detected during our earlier work; but Plant A operates in a batch mode and does not consistently discharge the same mix of waste chemicals.

An interesting case can be developed for several of the multi-chlorinated aromatic compounds (compounds 14-17): tetrachlorostyrene, hexachloroethylbenzene, DDE, and dichlorobenzophenone. None of these compounds are produced commercially; however Plant A did manufacture the pesticide 1,1-bis(p-

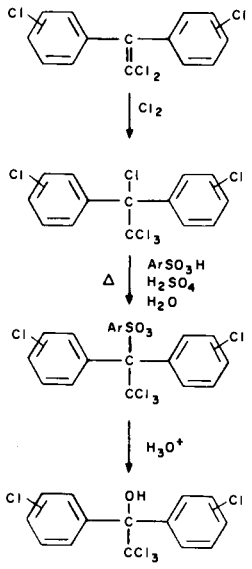

Fig. 5. Reaction pathway for the commercial production of 1,1-bis (p-chlorophenyl)-2,2,2-trichloroethanol; U.S. Patent 2,812,362 (1957).

chlorophenyl)-2,2,2-trichloroethanol. This pesticide is produced commercially using the reaction scheme outlined in Fig. 5. DDE is the unreacted starting material; tetrachlorostyrene, and hexachloroethylbenzene are probably cleavage by-products formed during the initial chlorination step or from the reaction intermediate 1,1-bis(p-chlorophenyl)tetrachloroethane; and dichlorobenzophenone could form during alkaline hydrolysis of either the tosylate ester intermediate or the pesticide itself. Two other structurally related compounds, bis(chlorophenyl) methanol (compound 44) and chlorophenylphenylmethanol (compound 67) first appear in the Northeast influent and effluent water, respectively. We think that these are probably degradation products of one of the above chlorinated species. We should point out that the pesticide itself was not detected in any of the wastewater or river water samples.

Although some of the methyl substituted (compounds 28-34) and chlorinated aromatics (compounds 21-24) and the solvent isophorone (compound 38) first appear in Plant A's waste effluent, they are common industrial chemicals which could also be entering the water system at various other points. This was confirmed by comparing concentration data for these compounds with the same data for the compounds specific to Plant A. The former compounds show much smaller changes in concentration between sampling locations suggesting multiple discharge sources.

Most of the compounds which appear for the first time in the Northeast treatment plant's influent (compounds 39-63) are common industrial or municipal contaminants. N(n-butyl)-benzene sulfonamide (compound 63) is interesting because it has not been identified in environmental samples before. Its major commercial use is as a plasticizer for polyamide materials. It has also been patented as a starting material in the production of sulfonyl carbamate herbicides (Stephens, 1976). The exact source of this contaminate is not yet known.

Those compounds originally appearing in the treatment plant's effluent water (compounds 64-68) were, of course, not present in the influent water; this suggests that they were formed during the treatment process. The most striking example is the polyethylene glycol derivative, tetraethyleneglycol-dimethacrylate (compound 66). This particular chemical is commonly used as a copolymer in many synthetic materials (Miller, 1971). It seems possible that a polymer entering the Northeast treatment plant is being degraded to monomer units during treatment. This compound was the most abundant chemical discharged in the Northeast treatment plant effluent; this leads to correspondingly high river water values. The di- and triethyleneglycol homologs

(compounds 64 and 65) were also identified.

Compounds first appearing in the river water (compounds 69-79) may be categorized into three groups according to source. First, those entering the river system from other industrial discharges such as various ethyleneglycol derivatives (compounds 69-71) and the plasticizers. Second, those compounds formed by the natural biological activity in the river, for example, chlorophyll (compound 76). Lastly, compounds which enter the river *via* rainwater runoff, most notably the herbicide dimethyl-2,3,5,6-tetrachloroterphthalate (compound 72).

In the finished drinking water a series of halogenated compounds appear which were previously undetected. It seems logical that these compounds, especially the halogenated phenols (compounds 81-84) are formed during the chlorination process.

Movement of Compounds Through the System. Comparisons among the columns of Table 2 indicate several trends. Large changes in concentration (up to four orders of magnitude) were observed between Plant A's effluent and the finished drinking water. Obviously, this large decrease in organic concentration is important when considering allowable discharge levels and treatment processes. For most compounds, the greatest concentration decreases occured between Plant A's effluent and the Northeast Treatment plant's influent (site a and b) and between the Northeast Treatment plant's effluent and the first upstream river sampling location (sites c to d). It is interesting that these large decreases in concentration are caused solely by dilution. In the first case, Plant A's effluent was diluted with other industrial waste water; in the second case, the municipal waste effluent was diluted with river water. In the two cases where treatment was performed, namely between the Northeast Treatment plant's influent and effluent (sites b to c) and between the Torresdale drinking water plant's influent and effluent (sites g to h), only small concentration decreases occurred. Thus, dilution is the most effective treatment process observed here.

On the other hand, the data in Table 2 show that there are several compounds where treatment processes, especially at the Torresdale plant are effective. These include the hydrocarbons, sterols, palmitic and stearic acids, some of the ethylene glycol compounds, the phenols, and chlorophyll. Unfortunately, it appears that the compounds of greatest environmental significance and biological activity are the least effected by the waste treatment processes.

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TABLE 2. Compounds and Their Concentration (ppb) Observed at the Various Sampling Sites (see Figure 1).

		Plant A	NE in	NE out	RM 106	RM 108	Tor in	Tor out	RM 118
I.	Plant A								
	A. Phenols								
1.	phenol	7000	60	20	0.3	0.3	[a]t	t	[b]-
2.	cresols	50	[c]un	20	-	-	-	-	-
3.	octyl phenols[d]	5000	400	200	3	2	0.4	.01	-
4.	nonyl phenols[e]	600	un	40	1	.02	.02	-	-
5.	4-octyl-2,6,di-t-butyl phenol	200	un	un	-	-	-	-	-
	B. Ethylene Glycol Derivatives								
6.	1,2-bis(2-chloroethoxy)ethane	100	un	un	1	1	t	t	-
7.	2-(p-1',1',3',3'-tetramethylbutylphenoxy)ethanol*	200	50	10	un	un	un	.02	5
8.	2-[2-(p-1',1',3',3'-tetramethylbutylphenoxy)ethoxy]ethanol*	100	un	un	0.6	0.3	un	.02	5
9.	2-(2-[2-(p-1',1',3',3'-tetramethylbutylphenoxy)ethoxy]ethoxy)ethanol*	400	un	un	0.4	0.2	un	.002	t
10.	2-[2-(2-[2-(p-1',1',3',3'-tetramethylbutylphenoxy)ethoxy]ethoxy)ethoxy]ethanol*	200	un	un	un	[f]NA	NA	NA	-
11.	2-(2-[2-(2-[2-(p-1',1',3',3'-tetramethylbutylphenoxy)ethoxy]ethoxy)ethoxy]ethoxy)ethanol*	un	un	NA	NA	NA	NA	NA	NA
12.	1-chloro-2-[2-(p-1',1',3',3'-tetramethylbutylphenoxy)ethoxy]ethane*	2000	200	70	0.6	0.5	0.3	0.2	-
13.	1-chloro-2(2-[2-(p-1',1',3',3'-tetramethylbutylphenoxy)ethoxy]ethoxy)ethane*	1500	120	50	0.6	0.4	0.3	0.2	-
	C. Chlorinated Compounds								
14.	tetrachlorostyrenes[e]	400	60	20	0.5	.06	-	-	-
15.	hexachloroethylbenzene[g]	un	un	-	-	-	-	-	-
16.	dichlorobenzophenones[g]	1000	110	60	1	0.2	0.1	0.1	-
17.	1,1-bis(chlorophenyl)-2,2-dichloroethylene (DDE)	1800	200	30	0.4	0.3	un	un	-
18.	2,5-dichloro-N(1,1-dimethyl-2-propynl)benzamide*	500	40	20	0.4	0.2	.02	.01	-
19.	chloro-N-(1,1-di-isopropyl)benzamide[g]*	50	t	t	t	t	-	-	-
20.	dichloro-N(1,1-di-isopropyl)benzamide[g]*	un	un	un	0.2	.06	.04	.02	-
21.	dichlorobenzenes[e]	100	100	t	[h]v	v	v	v	v
22.	chlorotoluene[g]	un	t	t	v	v	v	v	v
23.	trichlorobenzenes[e]	200	20	10	t	t	-	-	-

		Plant A	NE in	NE out	RM 106	RM 108	Tor in	Tor out	RM 118
24.	tetrachlorobenzenes[e]	200	t	t	t	t	-	-	-
	D. Plasticizers								
25.	di(2-ethylhexyl)adipate	2000	90	10	0.2	.04	.02	.002	-
26.	dioctylsebacate	200	-	-	-	-	-	-	-
27.	tri(t-butyl)phosphate	50	un	un	0.5	0.4	0.3	0.4	0.8
	E. Hydrocarbons								
28.	C_2 benzenes[e]	1000	100	v	v	v	v	v	v
29.	C_3 benzenes[e]	un	40	10	2	0.6	-	-	-
30.	naphthalene	un	20	4	t	t	t	-	-
31.	methyl naphthalenes	500	un	0.4	0.2	.02	t	t	t
32.	C_2 naphthalenes[e]	t	t	t	t	t	t	-	t
33.	C_3 naphthalenes[e]	un	t	t	t	t	t	-	t
34.	C_4 benzenes[e]	un	200	40	2	t	-	-	-
35.	$C_{14}H_{28}$[i]	200	10	un	-	-	-	-	-
36.	$C_{16}H_{32}$[i]	2000	30	10	-	-	-	-	-
	F. Others								
37.	binaphthyl sulfones[e]	0.6	un	un	un	-	-	-	-
38.	isophorone	un	100	10	3	0.6	t	t	-
II.	Northeast Influent								
	A. Phenols								
39.	phenyl phenol	-	un	un	un	un	-	-	-
40.	cumyl phenol	-	t	t	0.3	.01	.01	-	-
41.	t-butylmethoxy phenol	-	t	t	t	t	t	t	t
	B. Chlorinated Compounds								
42.	dichlorophenols[e]	-	un	0.4	0.4	t	t	un	-
43.	trichlorophenols[e]	-	t	0.1	0.1	t	t	un	t
44.	bis(chlorophenyl)methanol[g]	-	3	5	0.7	0.2	0.1	.002	-
	C. Plasticizers								
45.	triphenylphosphate	-	16	2	0.3	0.2	0.2	.03	t
46.	dibutylphthalate	-	50	25	0.6	0.4	0.1	0.1	0.3
47.	butylbenzylphthalate	-	40	100	0.6	0.3	0.3	0.1	0.4
48.	di(2-ethylhexyl)phthalate	-	200	100	1	1	0.5	0.6	0.5
	D. Hydrocarbons								
49.	pyrene	-	t	t	t	t	t	-	t
50.	fluoranthene	-	t	t	t	t	t	-	t
51.	anthracene	-	t	t	t	t	t	-	t
52.	phenanthrene	-	t	t	t	t	t	-	t

		Plant A	NE in	NE out	RM 106	RM 108	Tor in	Tor out	RM 118
53.	methylphenanthrene	-	t	t	t	t	t	-	t
54.	chrysene	-	t	t	t	t	t	-	t
	E. Others								
55.	cholesterol	-	400	200	2	1	0.6	-	0.9
56.	cholestanol	-	600	300	3	2	1	-	0.9
57.	α-terpineol	-	80	80	-	-	-	-	-
58.	2-phenyl-2-propanol	-	70	70	2	0.5	-	-	-
59.	stearic acid	-	h[j]	h	h	h	h	-	k[m]
60.	palmitic acid	-	h	h	h	h	h	-	m
61.	benzil	-	t	un	0.4	0.2	un	.02	-
62.	bornyl acetate	-	100	50	1	1	0.1	.002	-
63.	N(n-butyl)-benzene sulfonamide	-	un	un	0.6	0.3	t	t	-
III.	Northeast Effluent								
	B. Ethylene Glycol Derivatives								
64.	diethyleneglycol-dimethacrylate*	-	-	10	0.2	t	t	-	-
65.	triethyleneglycol-dimethacrylate*	-	-	35	0.5	0.1	5	-	-
66.	tetraethyleneglycol-dimethacrylate*	-	-	700	10	3	0.5	.02	-
67.	chlorophenylphenylmethanol[g]	-	-	t	0.1	0.1	0.1	un	-
	F. Others								
68.	menthol	-	-	8	un	-	-	-	-
IV.	River								
	B. Ethylene Glycol Derivatives								
69.	bis(2-[2-(n-butoxy)ethoxy]ethoxy) methane	-	-	-	un	1	2	t	3
70.	triethyleneglycol-di(2-ethylhexanoate)	-	-	-	0.2	0.1	-	-	0.1
71.	tetraethyleneglycol-di(2-ethylhexanoate)	-	-	-	2	3	t	-	2
	C. Chlorinated Compounds								
72.	dimethyl-2,3,5,6-tetrachloroterphthalate	-	-	-	un	un	un	.03	t
	D. Plasticizers								
73.	2,2,4-trimethylpentane-1,3-diol-1-isobutyrate	-	-	-	0.2	0.2	t	t	-
74.	2,2,4-trimethylpentane-1-3-diol-3-isobutyrate	-	-	-	0.2	0.3	t	t	-
75.	2,2,4-trimethylpentane-1,3-diol-di-isobutyrate	-	-	-	0.5	0.3	t	t	-

		Plant A	NE in	NE out	RM 106	RM 108	Tor in	Tor out	RM 118
	E. Others								
76.	chlorophyll[1]	-	-	-	2	7	4	-	9
77.	fluorenone	-	-	-	.04	.02	un	un	t
78.	ethylthiopyridine[g]	-	-	-	t	t	t	t	t
79.	1,1-bis(chlorophenyl)-2,2,2-trichloroethane (DDT)	-	-	-	t	t	t	un	-
V.	Drinking Water								
	C. Halogenated Compounds								
80.	dichloroisopropenyl toluene[g]	-	-	-	-	-	-	un	-
81.	bromochlorophenol[g]	-	-	-	-	-	-	un	-
82.	dibromophenol[g]	-	-	-	-	-	-	un	-
83.	dichlorobromophenol[g]	-	-	-	-	-	-	un	-
84.	dibromochlorophenol[g]	-	-	-	-	-	-	un	-

Notes for Table

a. t indicates that only trace levels were detected.
b. - indicates not detected.
c. un indicates that compound was not resolved gas chromatographically and, therefore, was not quantitated.
d. The predominant species was p-1,1,3,3-tetramethylbutylphenol although other isomers were present.
e. several isomers present.
f. NA indicates that analysis for these compounds was not carried out (not analyzed).
g. isomer unknown.
h. v indicates volatile compound; these compounds would not be retained in the water column during the summer months.
i. structure unknown, MW from CI, present in hexane fraction.
j. h indicates very high concentrations; these compounds give broad unresolved peaks which could not be quantitated.
k. m indicates moderate concentration.
l. chlorophyll was observed as a series of phytadienes.

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Industrial Wastes as a Source of Chemicals Entering the Environment

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ABSTRACT

We are studying the identities of organic chemicals which are introduced into the environment with industrial wastewaters. This is a report on an intensive study of a small, specialty chemicals plant and on its interaction with its receiving water system (a small river and estuary). Many compounds which had not been observed in the environment before were identified in this wastewater and river. The strategy for interpreting the data which led to these identifications will be presented. It is based on the synergistic use of electron impact and chemical ionization mass spectrometry, gas chromatography, high pressure liquid chromatography, high resolution mass spectrometry, and other circumstantial information.

INTRODUCTION

A detailed study on the identification of the organic compounds in the wastewater, receiving water, and receiving sediments from a specialty chemicals manufacturing plant has already been reported (Jungclaus, Lopez-Avila, Hites, 1978). Since that publication many other compounds have been identified. This paper is a summary of our interpretive strategy leading to these new findings.

EXPERIMENTAL

Wastewater and river water samples were collected in 1 gal amber glass bottles with teflon lined caps. To minimize biological degradation and to start the extraction, 200-300 ml of Nanograde dichloromethane (Mallinckrodt) were added immediately after sampling. Extraction was continued, after arriving in the laboratory, on a magnetic stirrer at pH=2 to separate acids and neutrals and at pH=10-11 to separate basic compounds.

Eight sediment cores were collected from the river by divers with a 6 cm ID x 80 cm stainless steel sampler. The cores were sectionated into 3 cm layers which were kept frozen in glass jars until analyzed. Soxhlet extraction of the wet sediment with isopropanol removed almost all of the organic compounds adsorbed onto it. A further extraction with benzene was performed but found to be unnecessary since no organic compounds related to the chemical plant were detected. Other details about experimental procedures can be found

elsewhere (Jungclaus, Lopez-Avila, Hites, 1978)

Gas chromatographic analyses were carried out on a Perkin Elmer 900 gas chromatograph equipped with a flame ionization detector and on a Perkin Elmer Sigma 1 instrument equipped with flame ionization, nitrogen-phosphorus, and flame photometric detectors. Both packed columns (180 cm x 2 mm ID glass column packed with 3% SP-2100 on Supelcoport) and capillary columns (25 m x 0.25 mm ID open tubular column coated with SE-52) were used. Mass spectra were obtained with a Hewlett-Packard 5982A GC/MS system with a dual EI/CI source, interfaced to a 5933A data system operating in a continuous scanning mode.

High pressure liquid chromatographic analyses were performed on a Waters Model ALC/GPC 204 liquid chromatograph equipped with two Model 6000 A pumps, a Model 440 dual absorbance detector and a Model 660 solvent programmer. A reverse phase packing (μ-Bondapak C_{18}, 10μ, Waters, in a 3.9 mm ID x 30 cm column) was used. The HPLC operational parameters for the industrial wastewater and sediment extracts were: gradient elution for 20% to 100% acetonitrile in water; flow rate 2 ml/min; ambient temperature; detector wavelengths 254 and 405 nm; detector sensitivity 0.1 to 0.5 AUFS. When industrial wastewater was pumped through the HPLC column, the gradient started with pure water. Collections of HPLC effluents were made using 5 ml pear shaped flasks; the samples were evaporated to dryness on a rotavapor, redissolved in dichloromethane, and transferred to a capillary tube which was introduced into the mass spectrometer via the direct probe. The temperature of the probe was gradually increased according to the volatility of the sample.

RESULTS AND DISCUSSION

The identification of organic compounds in the different environmental compartments was made possible only by the application of several analytical techniques. Very high concentrations of a few compounds hindered the identification of many of the minor peaks seen during GC/MS analysis. This dynamic range problem was partially solved by the use of high pressure liquid chromatography. Additional information for mass spectral interpretation was obtained from methane chemical ionization (CI) and from high resolution mass spectrometry. When an elemental composition was available from high resolution mass spectrometry, the EPA TSCA list (U.S. Environmental Protection Agency, 1977) and Chemical Abstracts were searched for related compounds. Whenever possible, model compounds were synthesized for the confirmation of hypothesized structures. A few interesting mass spectra have been selected for discussion here.

The identification of phenyl substituted naphthylamines (I, II) from their electron impact mass spectra alone was very difficult due to the lack of fragmentation. The electron impact spectrum of I (Fig. 1) shows an intense ion at m/e 260; the elemental composition of this ion obtained by high resolution mass spectrometry is $C_{19}H_{18}N$. However, the GC retention time was indicative of a higher molecular weight compound. A mass chromatogram indicated that the ion at m/e 331 might be the molecular ion, and an elemental composition $C_{24}H_{29}N$ was assigned for it from the high resolution mass spectrometric data. No other peaks in the electron impact mass spectrum were helpful in the structure elucidation because of their low abundance. Methane CI confirmed the molecular weight and also provided additional information because the fragment ions at m/e 220 and m/e 248 were seen previously in the methane CI mass spectrum of N-phenyl-naphthylamine (see Figure 2). The pro-

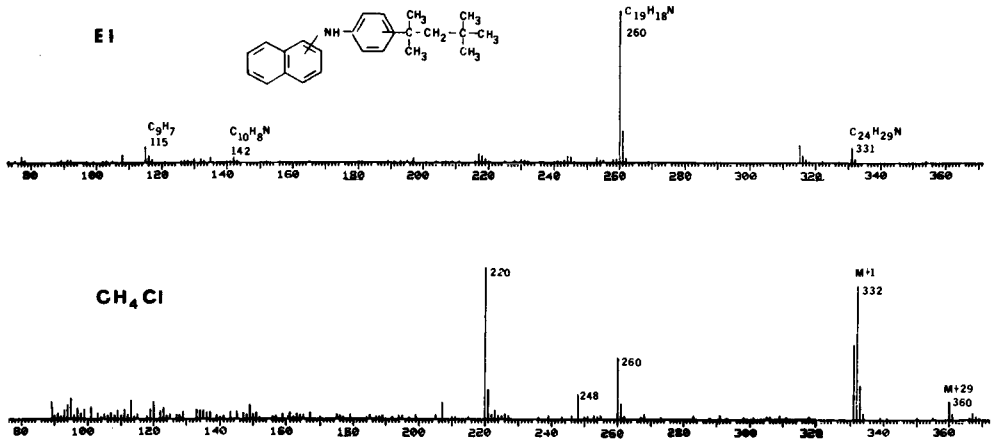

Fig. 1. Top: Electron impact mass spectrum of N-(t-octylphenyl)-naphthylamine (I).
Bottom: Methane chemical ionization mass spectrum of N-(t-octylphenyl)-naphthylamine (I).

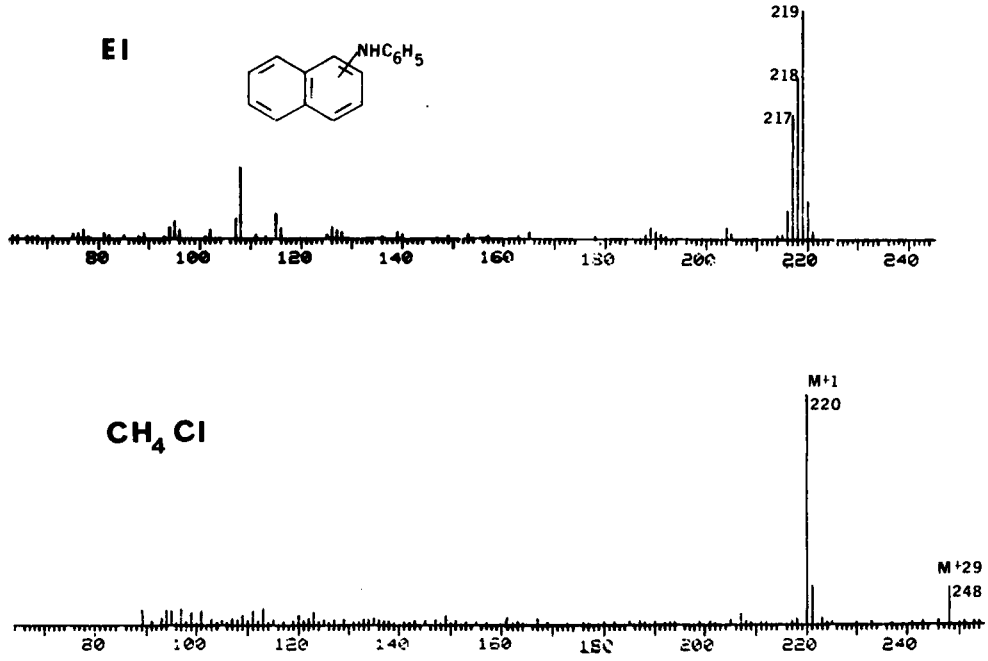

Fig. 2. Top: Electron impact mass spectrum of N-phenyl naphthylamine (II).
Bottom: Methane chemical ionization mass spectrum of N-phenyl naphthylamine (II).

posed structure is N-(t-octylphenyl)-naphthylamine (I). The loss of 71 amu (C_5H_{11}) from the molecular ion was indicative of a branched alkyl substituent; in addition, the ions at m/e 217, 218, 219 were characteristic for a phenyl naphthylamine.

A similar approach was used in the interpretation of another unknown compound (elemental composition $C_{20}H_{21}N$) which by electron bombardment formed a fragment ion at m/e 260 (elemental composition: $C_{19}H_{18}N$). The hypothesized structure was t-butylphenylnaphthylamine (II). Chemical Abstracts and the EPA TSCA list (U.S. Environmental Protection Agency, 1977) were searched for compounds having these elemental compositions to see whether or not they are currently manufactured. Unfortunately, they are not in production. Therefore, a model compound N-(n-butylphenyl)-2 naphthylamine (III) was synthesized and used to confirm the hypothesized structures.

Compound V gave a spectrum very similar to that of iminostilbene (IV). The electron impact mass spectrum (Fig. 3, top) shows an intense ion at m/e 193 with less intense ions at m/e 192, 191, 165 and 190. Because of the background ions, not unusual in the electron impact mass spectra obtained from very complex mixtures, the ion at m/e 236 could easily have been missed and the compound identified as iminostilbene. Its GC retention time, however, was different from that of iminostilbene and thus indicated a higher molecular weight compound. Methane CI (Fig. 3, middle) provided additional confirmation of the molecular weight. An elemental composition of $C_{16}H_{16}N_2$ was established by high resolution mass spectrometry. The final clue to its identification was provided by the similarity of its methane chemical ionization mass spectrum with that of 5-(3-dimethylaminopropyl)-10,11-dihydrodibenz-[b,f]azepine(VI) which was also present in our samples.

The mass spectrum of a sulphur containing compound is shown in Fig. 4; the identification of this compound was the most challenging since its source was not known (the compound had not been found in any of the wastewaters analyzed). The compound is environmentally persistent and concentrations ranging from 30 to 500 ppm were measured in the river sediment. The ion at m/e 263 seemed to be the molecular ion, and methane CI confirmed it. The fragment ion at m/e 106 is very common for alkyl anilines and pyridines so the elemental composition C_7H_8N established for this ion by high resolution mass spectrometry was not unexpected. Chemical ionization mass spectrometry provided additional information about the other end of the molecule since the ion at m/e 95 is very likely to be a protonated phenol. Furthermore, the loss of a protonated phenol fragment from the protonated molecular ion in CI would account for the ion at m/e 170. A mass chromatogram indicated a weak fragment ion at m/e 198 which was formed by a loss of 65 amu (SO_2H) from the molecular ion (see Fig. 4, top). A combination of these fragments indicates that the compound is VII. This structure (toluidine-sulfonyl phenol (VII)] based on the interpretation of both the electron impact and methane chemical ionization mass spectra, is also consistent with the similar behaviour under electron impact and chemical ionization of model compounds such as 4,4'-sulfonyl-diphenol and 4,4'-sulfonyl-dianiline.

ACKNOWLEDGEMENT

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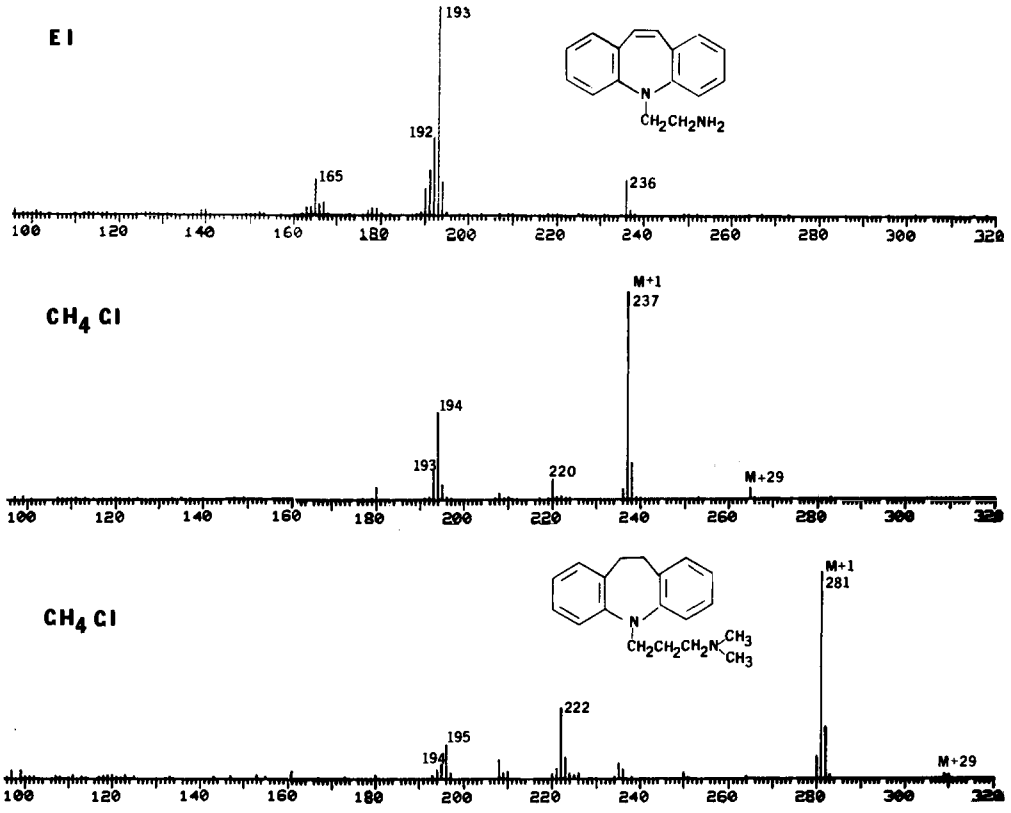

Fig. 3. Top: Electron impact mass spectrum of 5(2-aminoethyl)-dibenz[b,f] azepine (V). Middle: Methane chemical ionization mass spectrum of 5(2-aminoethyl)-dibenz[b,f]azepine (V). Bottom: Methane chemical ionization mass spectrum of 5(3-dimethylaminopropyl)-10,11-dihydrodibenz[b,f]azepine (VI).

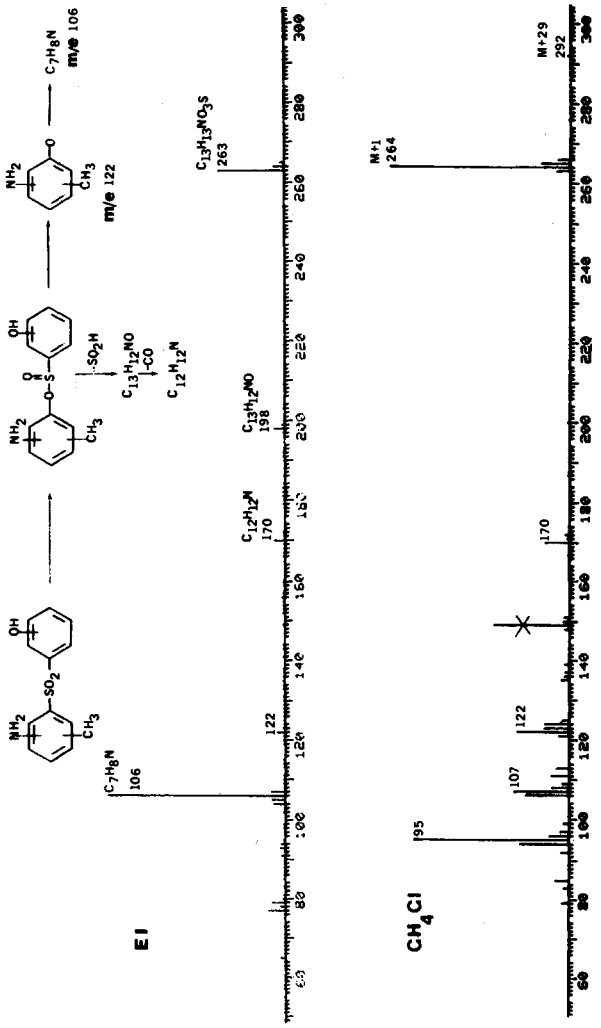

Fig. 4. Top: Electron Impact Mass Spectrum of toluidine sulfonyl phenol (VII).
Bottom: Methane chemical ionization mass spectrum of toluidine sulfonyl phenol (VII).

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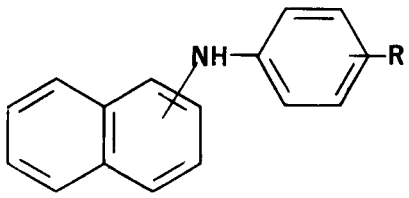
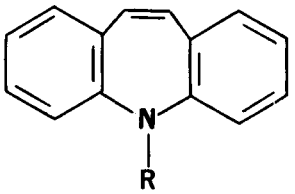


I R = $-C(CH_3)_2-CH_2-C(CH_3)_3$

II R = $-C(CH_3)_3$

III R = $-CH_2CH_2CH_2CH_3$

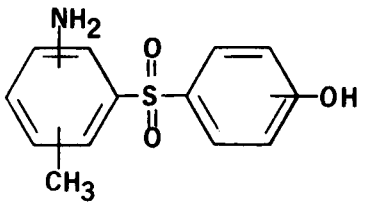


IV R = H

V R = $CH_2CH_2NH_2$

VI R = $CH_2CH_2CH_2N(CH_3)_2$

saturated at C_{10-11}



VII

Environmental Analysis from an Industrial Point of View

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ABSTRACT

In this lecture some conflict areas between industry and natural environment are discussed. Several industrial contributions to environmental analysis are mentioned. Requirements for a pre-market evaluation of chemical products are presented and a plea is made for a good use of the available resources and for regulations that reduce the hazards without taking away the benefits. Keywords: environmental analysis, pre-market testing.

THE RELATION BETWEEN INDUSTRIAL PRODUCTION AND NATURAL ENVIRONMENT

The introduction of industrial processes has brought about great changes in the appearance of the world during the last century. The positive effects of this development are well known: industry offers employment and essential (sometimes even luxury) goods to a tremendous number of people. Industrialization has greatly augmented the national income of many nations and has thus permitted the introduction of rather expensive social security systems. Industrial production of medicine has contributed to a much longer expectation of life and consequently to a dramatic increase of the population, especially in the neighbourhood of factories of which the production capacities were more and more scaled up. The environmental impact of these developments was hardly taken into account and today we all know how this unlimited optimism about the resilience of nature and the complete unawareness of the subtile and coherent processes in ecology has resulted in an alarming deterioration of nature. Especially the high rate of the decay has shocked us all and it is quite understandable that the ecological movement in the industrialized countries showed an almost exponential growth. Industry became an excellent target to concentrate activities on, for factories - notably the chemical ones - were clearly distinguishable, especially by their smoking chimneys and sometimes coloured effluent streams. Initially industrial management, not being familiar with this kind of action, reacted rather negatively to

the ecological movement and thus contributed towards a deepening of the gulf between industry and ecological action groups. During the discussions it was - and is - often forgotten that there are so many people working in industry that it is very improbable that the average worker will differ greatly from people outside industry in his attitude towards the natural environment. For he is drinking the same water, inhaling the same air, swimming in the same river. And he certainly does not like the idea of being the polluter during working hours and the victim of pollution at night. The difference between industrial people and others is that the former consider that they should also see to it that their industrial activities are continued. Not only in view of their economic interests, but also because they know they produce many useful and even essential products. Therefore industry has made important contributions in recent years to the reduction of environmental damage and it will continue to do so. However, it will also press for regulations to be based on relevant data and not merely on suspicions or emotions. Consequently, adequate environmental analyses are considered to be of great importance from an industrial point of view.

Where do we need these environmental analyses in industry ? Schematically, industrial production is the conversion of raw materials and energy into industrial products and - to some extent - into waste. Not only the waste but also the product may end up in the environment whether during or after use. The environmental impact of the winning of raw materials cannot be directly influenced by industry. The more so the impact on the environment of production and products.

ENVIRONMENTAL ANALYSIS IN THE CONTROL OF WASTE STREAMS

The reduction or even elimination of waste streams entering the air, water or soil has been a major topic in industry over the last few years. To achieve this, large numbers of environmental analyses had to be made. Analyses of all kinds are quite usual in industry, since many many modern processes cannot be run without an adequate set of analyses as a basis for their control. It is evident that extensive analyses had to be performed before process changes could be made to reduce the amount of effluent or before purification units could be constructed.

The analysis of waste streams is not an easy job, for effluents may vary very strongly in composition as well as in concentration. In addition - like all analyses in industry - the analytical techniques had to be judged on their costs and reliability. Yet impressive results have been obtained in a relatively short time thanks to the enthusiasm and the craftmanship of the analytical specialists in industry. To illustrate this, a set of monitors (for mercury and active chlorine) are shown which are in use now to steer a treatment plant of a mercury-containing effluent in a chlorine-alkali industry.

Many analytical techniques had to be developed to measure air pollution. In immission measurements additional problems were encountered, viz. the low concentrations concerned and the influence of the weather conditions on the results. These problems have been solved by the development of highly versatile

mobile laboratories carrying special monitors as well as more universally applicable instruments like multidetector GLC and the necessary meteorologic and logistic equipment (Verhoeven, 1978). Fewer problems are encountered in measuring within factories, although the centralized monitoring of vinylchloride on the 1 ppm level at several points within and around a production unit includes a large amount of sophisticated analytical skill. Many valuable contributions to the development of reliable analytical techniques for environmental analysis were obtained through joint efforts of related industries. In some cases governmental and university laboratories have been cooperating as well. The exchange of knowledge and a great variety of ring tests have resulted in many standard tests as a basis for action. Examples of these cooperations are:

- the development of standard biodegradability tests for anionic detergents by the Hauptausschuss Detergentien in Western Germany (Fischer-Hussmann) which were the basis for the present OECD tests;
- the development of standard mercury analyses in different substrates by a working party of the BITC (1974, 1976a, 1976b);
- the development of analyses for chlorinated solvents by a BIT-SC working group (1976);
- the comparison of biodegradability tests for chemicals by a CEFIC working group.

It should be mentioned that in addition to analytical chemical techniques other methods may be helpful in the detection and analysis of pollutants. In some cases they may even be better. An example is the effort in our laboratory to find an analytical technique to measure the efficiency of a unit for the treatment of an evil smelling air flow from one of our factories: after some years about 200 compounds were isolated by GLC and partly identified by MS, but no correlation with the stench was found; the problem was solved in a few days by the introduction of a mobile olfactometric unit (of TNO-Apeldoorn, the Netherlands) (Stork, 1977; Logtenberg, 1978).

The development and introduction of new measuring techniques has greatly enhanced our knowledge of the qualitative and quantitative composition of industrial effluent streams. Many waste-reducing process improvements and purification units have been designed on the basis of these data. To all who are familiar with the subject it will be clear that this has not yet resulted in a complete control of all industrial effluents. Several additional factors play a role here. However, considerable progress has already been made and results are visible in many places.

ANALYSIS OF THE ENVIRONMENTAL IMPACT OF PRODUCTS

As mentioned earlier, the impact of industrial production on the natural environment is not limited to the emission of effluents. Effluent streams mainly contain by-products, and continuous efforts are made by industry to reduce the amount of these by-products in favour of the yield of the desired products. The products, however, also have a chance to enter the environment. This may either happen during use, as with propellants and

volatile organic solvents, or at the end of their life-time, as with cars, clothes, refrigerators, etc. Until some years ago little attention was paid to the behaviour of these materials in the environment and to their effect on natural systems. In this connection another factor has to be considered: most industrial products become outdated sooner or later; especially in the light of new developments they show shortcomings which may concern

- price
- performance
- safety
- environmental impact
- energy and/or raw material content.

As industry is not only producing and selling products, but also has the research and development facilities to innovate, there has been a constant replacement so far and a stream of new products has come into the market and - with some delay - into the environment. The demand for an evaluation of the potential environmental impact of new products is therefore quite logical, and it has been accepted by industry that such an evaluation before introduction is necessary. In several branches of industry these evaluations are actually made today.

Factors which may affect the environmental impact of a product are:

- identity
- chemodynamic properties
- production volume
- distribution
- biotic/abiotic degradability
- accumulability
- acute toxicity
- chronic toxicity (incl. DNA damage)
- eco-toxicity
- removability / detoxification.

It stand to reason that a considerable effort involving a great variety of analytical techniques will be required to produce these data. Some critical notes are to be made here: industrial innovation is a risk-bearing activity, considerable amounts of money are to be invested during the development period which may last several years. A delay in the introduction will greatly enlarge the amount of money to be invested. However, investments in new products are only made in relation to profit expectations. If test programs are becoming too expensive (directly or indirectly - via delay in introduction) this will result in a declining innovation. There are strong indications that this is already happening. Such a development will be desastrous to industrial R&D but also detrimental to society, as since the introduction of better, cheaper, safer and/or less polluting or energy-demanding products may be inhibited.

A test program for new products should therefore ensure a better knowledge of the chemicals involved <u>with the available resources</u>.

This will imply in practice:

- case-by-case treatment
- step-by-step testing
- careful selection of the substrate
- cooperation of all concerned / confidentiality.

Especially the human toxicity tests are expensive, and time-consuming, while the capacity of the qualified institutes is rather limited. Fortunately, the chemical analysis rarely proves to be the bottle-neck in costs or time. Nevertheless the chemical analyst contributing to these test programs should know his responsibilities !

SOME LEGAL ASPECTS

There has been strong pressure on governments in the last few years to control the introduction of new chemical products into the market by special legislation. This pressure is based on the assumption that it might still be possible for industry to start large commercial productions of new products without any control. A short study of the list of existing regulations in the field of safety and environmental protection will help to elucidate this point. For this list includes

- "Sectorial" laws to control the pollution of surface water, ground water, air and soil, hazardous waste and household waste disposal
- Occupational safety and health laws also covering hazardous plants and radioactive materials
- Product control laws on pharmaceuticals, pesticides, food and animal food additives, food packaging materials, cosmetics and detergents
- Product liability acts
- Environmental impact studies.

In view of this impressive list of laws, most of which originate from the last few years, it will be clear that it is practically impossible to start new initiatives without being confronted with one or more of these laws. The addition of still more laws involves a considerable risk of overlap and may be an extra obstruction to new developments. A tragic example is the paralysis of innovation in the field of pest control in the USA, where the development of new highly specific sophisticated pest control methods has been made impossible for years by the rigid enforcement of a new pesticide act (Tucker, 1978). It must therefore be emphasized that REGULATION (if any) SHOULD REDUCE THE HAZARD WITHOUT TAKING AWAY THE BENEFITS.

It should also be borne in mind that

- the consequences of the zero-alternative (doing nothing) may be worse
- a negative cannot be proved
- zero-risk does not exist.

If we disregard these self-evident truths, future generations may say "They were a bunch of ignorants and when, in the end, they came to their senses, they got hysterical" (Tsjechow).

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Design of the Organics Analysis in an Environmental Monitoring System

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ABSTRACT

Several recent United States Federal laws dealing with the environment require extensive measurements of the presence and concentration of a broad variety of toxic and persistent organic compounds. The concern for environmental pollution by organics expressed in these laws developed after the discovery of sensitive and reliable analytical techniques in the post World War II era. Because of the new legal requirements, the design of the organics analysis will become a far more significant aspect of an environmental monitoring system than it was in the past. A key aspect of the design of the organics analysis is the selection of the analytical methodology. Analytical method selection guidelines are presented and illustrated with the United States Environmental Protection Agency's, "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants."

KEYWORDS

Environmental Pollution
Analysis of Organics
Analytical Methodology
Gas Chromatography
Mass Spectrometry
Industrial Effluents
Toxic Compounds
Priority Pollutants

INTRODUCTION

In the United States the period beginning about 1970 will likely be described by legal historians as the great era of environmental law. Among the many recent pieces of Federal legislation dealing with the environment, several address in some detail the problem of pollution by toxic and potentially carcinogenic organic compounds. Major legislation in this category includes the 1972 amendments to the Federal Water Pollution Control Act (PL 92-500), the Safe Drinking Water Act of 1974 (PL 93-523), and the Toxic Substances Control Act of 1976 (PL 94-469). Among other requirements, these laws establish the need for extensive measurements of the presence and

concentration of a broad variety of organic compounds in a number of different sample types.

The major thrust behind the passage of the new legislation was the correlations of adverse health or ecological effects with measurements of the presence and concentration of a variety of pollutants. These measurements were made possible by significant advances in analytical instrumentation, electronics, computer science, and analytical chemistry during the 1960's and early 1970's. For the identification and measurement of organic compounds, major breakthroughs included the discovery of gas chromatography; the development of the electron capture, flame ionization, and other detectors; the application of the mass spectrometer as a detector in gas chromatography; the development of the quadrupole mass spectrometer; and the extensive advances in solid state microelectronics that made possible the relatively low cost digital computer. Today there is available an array of powerful tools for the measurement of organic pollutants and the future will undoubtedly provide more. For example, high performance liquid chromatography is an important new tool in many areas of analytical chemistry, and it should have many significant applications in environmental chemistry. The leaders of the environmental movement and those who are pleased with the general public concern for the environment should not forget the major contributions from analytical chemistry and the developers of new analytical methods. On the other hand, it is ironic that environmental regulation may often impact heavily certain organizations that made significant contributions to the advances in analytical techniques.

SELECTION OF ANALYTICAL METHODS

With the large number of measurement techniques available, the designer of an environmental monitoring system must select those most appropriate for the situation. Although many tools exist, each has a different capability, cost, and complexity of operation. This section is intended to present general guidelines for the selection of analytical methodology for organic compounds. The discussion will emphasize compounds that are sufficiently volatile for gas chromatography because these have been studied most during the last 20 years. However, the principles are general and apply to all types of organic compounds.

There are two general goals that affect the method selection process. The target compound (TC) goal is traditional in chemical analysis and is widely accepted in related sciences. The target compounds, whose concentrations are desired, are usually contained on a list submitted to the analytical laboratory. The list of target compounds affects method selection because with this goal, sample processing and measurement methods may be optimized for the target compounds. Detailed chemical-instrumental procedures may be designed to isolate the target compounds from the sample matrix and known interferences, concentrate the target compounds, and measure their concentrations with various general purpose or selective detectors. Laboratory control standards are usually employed to establish that isolation does occur, and to optimize the procedure for maximum recovery of the desired components and minimum interference from other components. Chromatographic separations are included in the optimization process.

The gas chromatography-electron capture detector procedures for chlorinated hydrocarbon pesticides are examples of the optimized target compound

approach. However, the justification for selection of a specific method requires more information than the decision to measure target compounds. The additional information needed is discussed after a presentation of the other general type of analytical goal.

The broad spectrum (BS) approach is in sharp contrast to the TC approach. The idea of the BS approach is to seek a broad spectrum picture of whatever is present in a sample as a major or minor component. This kind of analysis is not guided by a predetermined list of compounds to be measured. The BS approach has existed since the beginning of chemical analysis. However, the practical and economic pursuit of this goal was often not possible in the past. The development of the computerized gas chromatography-mass spectrometry system, and other similar technologies, has made this goal a feasible and desirable alternative for many types of samples.

With the BS approach, sample preparation is designed to be as simple as possible to preclude losses of significant sample components and to minimize the possiblity of sample contamination. The idea is to divide the sample into broad classes of compounds and to apply general purpose chromatographic methods for the separation of the compounds in each group. Literally hundreds of thousands of compounds can be potentially included in a broad class, but it is usually safe to assume that only a relatively few compounds are present in each class in most samples.

For the BS approach a spectroscopic detector is required. Spectroscopic detectors are defined as devices that continuously measure spectra of energy absorptions, ion abundances, etc. of various components as they elute from a chromatographic system. The spectra are of sufficient quality to permit identification of components without prior knowledge of which compounds are present. A gas chromatography-mass spectrometry (GC/MS) system operating in the continuous repetitive measurement of spectra mode is a spectroscopic detector, but a GC/MS system operating in the selected ion monitoring mode of data acquisition (Budde, 1977) is not. The very nature of the BS approach implies the need to generate information sufficient to recognize and identify the unexpected. The most beneficial result of the BS approach is the frequent discovery of significant but previously unrecognized pollutants.

If the decision is made to use the BS approach, method selection is restricted. Gas chromatography-mass spectrometry may be the only viable choice, although additional tools should become available in the future. On the other hand, if the decision is made to use the TC approach, careful consideration of the sample type is necessary before a method selection can be made. Environmental systems may be divided into three broad classes:

1. Systems of type 1 are relatively closed, i.e., there is some control of the entry of components into the system, and all components are well defined. An example is the output from a chemical plant that uses raw materials of known composition, processes them according to a particular procedure, and generates products and by-products that are well-defined.

2. Systems of type 2 are somewhat open in that entry of new components is possible but not frequent or likely, and the components are somewhat defined. An example is the output from a drinking water treatment plant that uses an uncontaminated ground water source, and chlorinates to produce a more or less constant variety of halogenated methanes.

3. Systems of type 3 are wide open to entry of almost anything at any time, and components are poorly defined. An example is the Mississippi River at St. Louis, Missouri.

For measurements with a type 1 environmental system and a TC analytical goal, reliable results may be obtained with a chromatographic method and a conventional general purpose or selective detector, e.g., flame ionization or electron capture. With this type of sample the probability of unexpected components is low. The optimized TC procedures assure that most or all potential interferences are eliminated and, if a selective detector is used, the effects of interfering substances are reduced further. Under these conditions the retention index may be a reliable method of peak identification. The often cited advantage of this approach is the potential economy of operation since the equipment employed is relatively simple, inexpensive, and easy to use by relatively low cost technicians. The principal disadvantage is that in order to include all environmentally significant compounds, literally hundreds of different procedures need to be developed, tested, and documented. The implementation of all these procedures for a defined but relatively complex sample would be slow, complex, and relatively costly.

With type 2 environmental systems and a TC goal, the probability of unexpected components increases. If these unexpected components are interferences that are not eliminated by the sample preparation, there is a clear possibility of erroneous results if a general purpose chromatographic detector is in use. For type 2 environmental systems a selective chromatographic detector or a spectroscopic detector is required. Microcoulometric, electrolytic conductivity, or flame photometric gas chromatographic detectors are selective and may be appropriate choices. A gas chromatography-mass spectrometry system operating in the selected ion monitoring mode of data acquisition (Budde, 1977) is a highly selective detector. However, for measurements of type 2 systems, it is recommended that a spectroscopic detector be used regularly to confirm a fixed percentage of the identifications and to analyze any samples that deviate from the normal pattern.

With a type 3 environmental system and a TC goal, the application of a spectroscopic detector is required. With the type 3 system the probability of unexpected compounds is high, and the qualitative information produced by conventional chromatography detectors is insufficient for reliable identifications. Again, as in the BS approach, the only viable tool may be gas chromatography-mass spectrometry (GC/MS). General purpose conventional chromatography detectors may have several important applications in support of the GC/MS methodology. These include prescreening to determine dilution requirements, to eliminate samples with no components above a given threshold, or to optimize chromatography operating parameters.

COST CONSIDERATIONS

Many decisions of method selection are greatly influenced by cost considerations. If cost considerations are used, the total cost of an analytical method should be used. This should include the following:

1. Equipment investment
2. Sample preparation costs
3. Instrumentation operating costs
4. Quality control costs

5. Equipment maintenance costs
6. Training and management costs

There is some indication that for measurements of relatively large numbers of target compounds, e.g., 40-60 or more, in type 1 or 2 environmental samples, broad spectrum type methods may be less costly per TC than chromatography methods that use conventional general purpose or selective detectors and optimized sample preparation procedures. Figure 1 shows plots of changing approximate (indicated by the broad bands) total costs as a function of the number of target compounds for a mass spectrometer and conventional detectors. With a small number of target compounds, total costs are dominated by equipment captial costs, and the mass spectrometer approach is relatively expensive. However, as the number of target compounds increases, the cost of the GC/MS approach remains relatively constant, while the costs of methods using conventional detectors increase significantly. There appear to be three general reasons for this trend:

1. Optimized TC sample preparation procedures are often quite rigorous and complex to assure that most or all potential interferences are eliminated, and these kinds of procedures must be employed with conventional chromatography detectors. With a mass spectrometer the sample preparation may often be much simpler, analogous to the BS approach, because the mass spectrometer output provides adequate information, in most cases, to reliably identify most components of the sample.

2. For a broad range of target compounds, a variety of conventional chromatography detectors and supporting equipment is required. This is because chromatography detectors are designed for some selectivity to further minimize the effects of interfering substances. As the number of target compounds increases so does their diversity, hence, the need for more equipment. On the other hand, the mass spectrometer is a universal detector which precludes interference by the very nature of its output. It can accommodate a variety of compound types without a significant change in operating procedures.

3. Conventional chromatography methods with general purpose or selective detectors often rely heavily on retention indices for the identification of compounds. This requires the rigorous control of operating conditions that could affect the retention index, e.g., flow rates, temperatures, temperature programming rates, etc. Measurements of control standards must be made at frequent intervals to assure that conditions are well controlled. With a GC/MS retention indices are not critical and careful control of operating conditions that affect them is not critical.

For monitoring a large number of target compounds encompassing a broad range of compound types, it appears that the additional cost of GC/MS instrumentation is offset by savings in other equipment and operating costs. Additional experience with all the methods discussed over the next few years will permit more exact quantitation of the cost-effectiveness of various approaches.

THE PRIORITY POLLUTANT PROGRAM

The U.S. Environmental Protection Agency's (EPA) priority pollutant program is an example of the application of the method selection guidelines. During 1976 the EPA was required to begin a program to establish for industrial

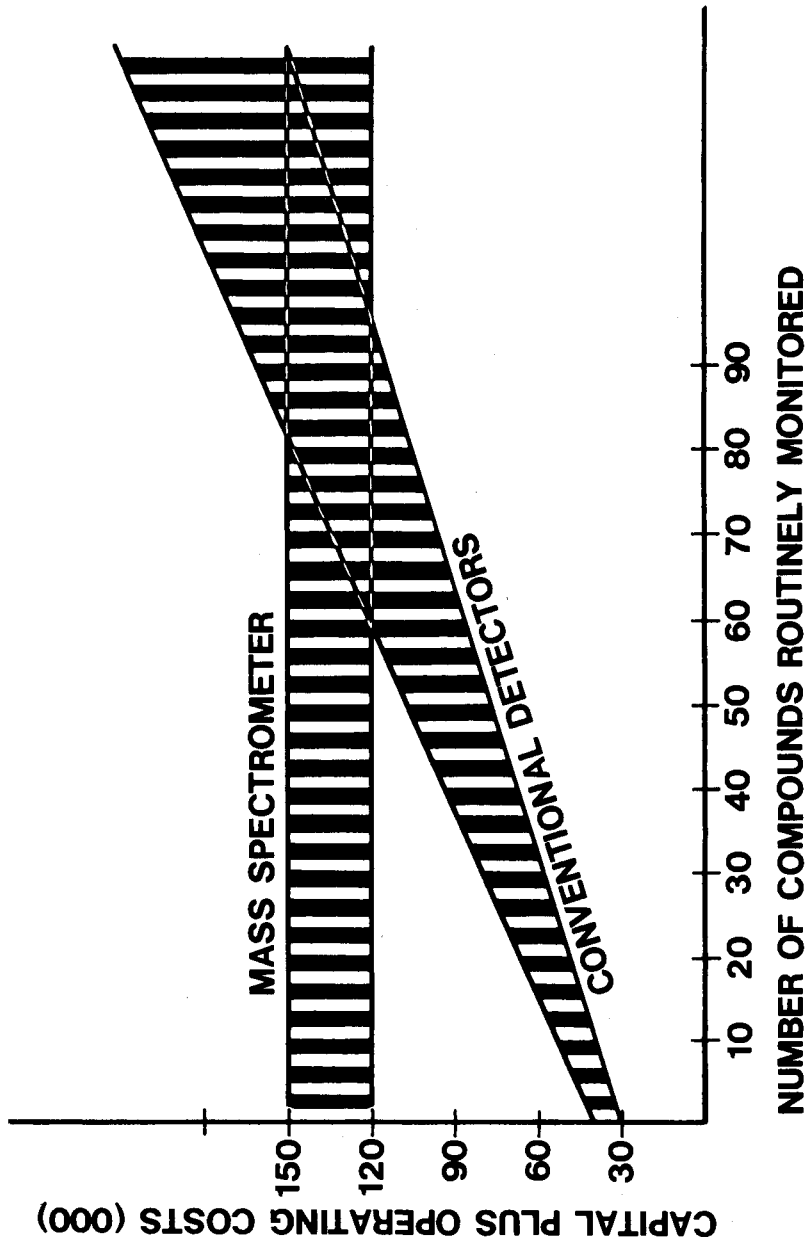

Fig. 1. Capital plus operating costs for mass spectrometer and conventional detectors as a function of the number of compounds routinely monitored.

wastewater effluent limitations for a group of 129 priority pollutants, and to recommend treatment technology to meet the limitations. The priority pollutants were selected on the basis of known human or animal toxic and carcinogenic effects. The 129 materials included 106 specific organic compounds, nine product formulations that are mixtures of organic compounds, twelve metals, cyanide ion, and asbestos. The first step in the process of establishing limitations was a major program to identify and measure these materials in various wastewaters from a number of industries.

Clearly the analytical goal of the organics analysis was a group of target compounds, and the samples were of type 3, i.e., uncharacterized with poorly defined components. Also a rather large number of target compounds was of interest, and these covered a range of chemical and physical properties. All of these factors pointed to the selection of broad spectrum sample preparation methods, extensive use of gas chromatography, and a spectroscopic detector. Gas chromatography - mass spectrometry was selected as the basis for the analytical protocol. The target compounds were divided into five broad classes as follows:

1. A group of 46 compounds isolated from a pH=11 adjusted sample by extraction with methylene chloride. These compounds are identified and measured by GC/MS and are listed in Table 1.

2. A group of 26 compounds and product formulations extracted from a sample at ambient pH with 15% methylene chloride in hexane. These compounds are shown in Table 2. They are initially detected by gas chromatography with an electron capture detector, but must be confirmed by GC/MS. There may be some overlap between this and the first fraction.

3. A group of 30 compounds isolated by the inert gas purge and trap procedure (Bellar, 1979), and identified and measured with GC/MS. These compounds are shown in Table 3, and there may be some overlap with the other fractions.

4. A group of 11 compounds extracted from a sample adjusted to pH=2. The solvent is methylene chloride and these compounds are shown in Table 4. The identification and measurement is by GC/MS.

5. Finally, two compounds, acrolein and acrylonitrile, are very water soluble and not easily isolated from aqueous samples. These are identified and measured by direct aqueous injecton GC/MS (Harris, 1974). These are also shown in Table 3.

In addition to the compound names, Tables 1-4 show the Chemical Abstract Service (CAS) Registry numbers. These unqiue identifiers are useful for computer storage and retrieval of results, and searching of related databases for information about a particular substance. The quantitative analysis by GC/MS includes an extensive quality control program to establish the precision and accuracy of the measurements in a variety of concentration ranges. A great deal of information about the precision and accuracy of these measurements should become available in the next few years.

TABLE 1 The Forty-six Compounds of the Base-Neutral Fraction

Compound Name	CAS Registry Number
1,3-Dichlorobenzene	541-73-1
1,4-Dichlorobenzene	106-46-7
1,2-Dichlorobenzene	95-50-1
Hexachloroethane	67-72-1
Bis(2-Chloroethyl) ether	111-44-4
Bis(2-Chloroisopropyl)ether	39638-32-9
N-nitrosodi-n-propylamine	621-64-7
Isophorone	78-59-1
Nitrobenzene	98-59-1
Hexachlorobutadiene	87-68-3
1,2,4-Trichlorobenzene	129-82-1
Naphthalene	91-10-3
Bis(2-Chloroethoxy)methane	111-91-1
Hexachlorocyclopentadiene	77-47-4
2-Chloronaphthalene	91-58-7
Acenaphthylene	208-96-8
2,6-Dinitrotoluene	606-20-2
Acenaphthene	83-32-9
Dimethylphthalate	131-11-3
Fluorene	86-73-7
4-Chlorophenyl phenyl ether	7005-72-3
2,4-Dinitrotoluene	121-14-2
1,2-Diphenylhydrazine	122-66-7
Diethylphthalate	84-66-2
N-Nitrosodiphenylamine	86-30-6
Hexachlorobenzene	118-74-1
4-Bromophenyl phenyl ether	101-55-3
Phenanthrene	85-01-8
Anthracene	120-12-7
Di-n-butylphthalate	84-74-2
Fluoranthene	106-44-0
Pyrene	129-00-0
Benzidine	98-87-5
Butylbenzylphthalate	85-68-7
Bis(2-ethylhexyl)phthalate	117-81-7
Chrysene	218-01-9
Benzo(a)anthracene	56-55-3
Benzo(b)fluoranthene	205-99-2
Benzo(k)fluoranthene	207-08-9
3,3'-Dichlorobenzidine	91-94-1
Di-n-octylphthalate	117-84-0
Benzo(a)pyrene	50-32-8
Indeno(1,2,3-cd)pyrene	193-39-5
Dibenzo(a,h)anthracene	53-70-3
Benzo(g,h,i)perylene	191-24-2
Nitrosodimethylamine	62-75-9

TABLE 2 The Twenty-six Compounds of the Pesticide Fraction

Compound Name	CAS Registry Number
β-endosulfan	33213-65-9
α-Benzenehexachloride	319-84-6
γ-Benzenehexachloride	58-89-9
β-Benzenehexachloride	319-85-7
Aldrin	309-00-2
Heptachlor	76-44-8
Heptachlor epoxide	1024-57-3
α-endosulfan	959-98-8
Dieldrin	60-57-1
4,4'-DDE	72-55-9
4,4'-DDD	72-54-8
4,4'-DDT	50-29-3
Endrin	72-20-8
Endosulfane sulfate	1031-07-8
δ-Benzenehexachloride	319-86-8
Chlordane	57-74-9
Toxaphene	8001-35-2
Aroclor-1242	53469-21-9
Aroclor-1254	11097-69-1
Aroclor-1221	11104-28-2
Aroclor-1232	11141-16-5
Aroclor-1248	12672-29-6
Aroclor-1260	11096-82-5
Aroclor-1016	12674-11-2
2,3,7,8-Tetrachlorodibenzo-p-dioxin	1746-01-6
Endrin Aldehyde	---

TABLE 3 The Thirty Compounds of the Purgeable Fraction and Two Direct Aqueous Analytes

Compound Name	CAS Registry Number
Acrolein	107-02-8
Acrylonitrile	107-13-1
Chloromethane	74-87-3
Dichlorodifluoromethane	75-71-8
Bromomethane	74-83-9
Vinyl chloride	75-01-4
Chloroethane	75-00-3
Methylene chloride	75-09-2
Trichlorofluoromethane	75-69-4
1,1-Dichloroethylene	75-35-4
1,1-Dichloroethane	75-34-3
Trans-1,2-Dichloroethylene	540-59-0
Chloroform	67-66-3
1,2-Dichloroethane	107-06-2
1,1,1-Trichloroethane	71-55-6
Carbon tetrachloride	56-23-5
Bromodichloromethane	75-27-4
Bis(chlormethyl)ether	542-88-1

1,2-Dichloropropane	78-87-5
Benzene	71-43-2
Trans-1,3-Dichloropropene	542-75-6
Cis-1,3-Dichloropropene	542-75-6
Trichloroethylene	79-01-6
Dibromochloromethane	124-48-1
1,1,2-Trichloroethane	79-00-5
2-Chloroethyl vinyl ether	110-75-8
Bromoform	75-25-2
1,1,2,2-Tetrachloroethene	127-18-4
Toluene	108-88-3
1,1,2,2-Tetrachloroethane	79-34-5
Chlorobenzene	108-90-7
Ethyl Benzene	100-41-4

TABLE 4 The Eleven Compounds of the Acid Fraction

Compound Name	CAS Registry Number
Phenol	108-95-2
2-Chlorophenol	95-57-8
2-Nitrophenol	88-75-5
2,4-Dimethylphenol	105-67-9
2,4-Dichlorophenol	120-83-2
p-chloro-m-cresol	59-50-7
2,4,5-Trichlorophenol	88-06-2
2,4-Dinitrophenol	51-28-5
4-Nitrophenol	100-02-7
4,6-Dinitro-o-cresol	534-52-1
Pentachlorophenol	87-86-5

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Automatic Analysis of Organic Pollutants in Water via a Calculator-controlled GC/MS

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ABSTRACT

A low cost microcomputer-controlled gas chromatograph/mass spectrometer (GC/MS) has been programmed to automatically carry out the routine analysis of water samples or extracts of water samples. This greatly simplifies the analysis and reduces the need for operator attention. Routine time consuming operations have been eliminated or reduced through computer control.

The U.S. EPA (1977) has defined 114 compounds as chemical indicators of organic pollution in water. These indicators are divided into two groups: 30 purgeable organic compounds and 84 semi-volatile and nonvolatile organic compounds. The latter compounds may be analyzed via liquid/liquid extraction and subsequent direct injection into a GC/MS. The purgeable compounds, may be separated from the water substrate by a technique known as vapor stripping (Bellar and Lichtenberg (1974)).

To carry out these analyses, the calculator-controlled GC/MS can be programmed to automatically tune itself and set up proper gas chromatograph and mass spectrometric conditions. The instrument will accept raw water samples for the analysis of purgeable organic compounds. Subsequent sampling, analysis and data reduction can be carried out under control of the calculator. It is thus possible to make GC/MS analysis of purgeable organic compounds in water semi-automatic. Indeed, the operator need only insert his water sample, and answer four questions asked by the calculator. The sampling process and analysis is completely controlled by the calculator. The analysis of non-purgeable organic compounds can be carried out in this manner, however the liquid/liquid sampling procedure has not been automated. A Final Report containing qualitative and quantitative results is automatically printed out at the end of each analysis.

Keywords: purge and trap, sewage, industrial effluent, drinking water

EXPERIMENTAL

A Hewlett-Packard 5992A GC/MS system was used for these analyses. Data was stored on a Hewlett-Packard 18939A Flexible Disc accessory storage device. A Hewlett-Packard 7675A Purge and Trap Sampler was used for the purgeable samples.

The extractions and GC parameter setups were executed according to the procedure outlined by the EPA. High purity grade methylene chloride was used as the extractant.

High purity grade helium was used as the purge gas. It was purged at a rate of 40 ml/min for 10 minutes. The purge volume was 60 ml of water. The trap desorption occurred with a flow of 20 ml/min of helium for 4 minutes at a temperature of 200°C.

RESULTS AND DISCUSSION

The analysis scheme is carried out in the following manner. The operator is queried as to which of the following analysis is needed:

Purgeable Organic Compounds
Acid Extract
Base/Neutral Extract
Pesticides

The calculator informs the operator of the proper GC column to be used for the particular analysis requested. The operator then is requested to enter the concentration range of the sample and the sample identification number. After this is completed, the calculator sets up the proper gas chromatographic and mass spectrometric conditions.

If a Purgeable Organic Analysis (POA) has been requested, the calculator will automatically initiate the sampling sequence for this analysis. The sampling sequence consists of a purge cycle and a trap desorb cycle followed by a trap vent cycle.

A stream of helium gas is bubbled through the water sample and then passed through a tube of adsorbent material, Tenax GC. Helium carries the volatile organics out of the water and into the adsorbent trap. The helium flow is then reversed through the trap and shunted into the GC/MS. The trap is heated rapidly to 200°C to drive the purgeable organics into the GC/MS system. The purgeable organic compounds pass through a GC column where they are separated prior to entering the mass spectrometer. A mass spectrum for each compound is then collected and stored on a magnetic disc. A total chromatogram is printed which displays the response of the instrument with time. A representative total ion chromatogram is shown in Figure 1.

A chromatogram reveals the presence of several compounds in the sample. Ten ppb of internal standard, 2-bromo-1-chloropropane, has been added to help determine accurate quantitation and relative retention times.

To aid in the identification of the unknowns, the mass spectrum representing the top of each GC peak is stored along with an appropriate background spectrum. The background spectrum is then subtracted from the unknown spectrum and the result can be displayed and then searched against a library of known spectra. Each peak can usually be identified in this way.

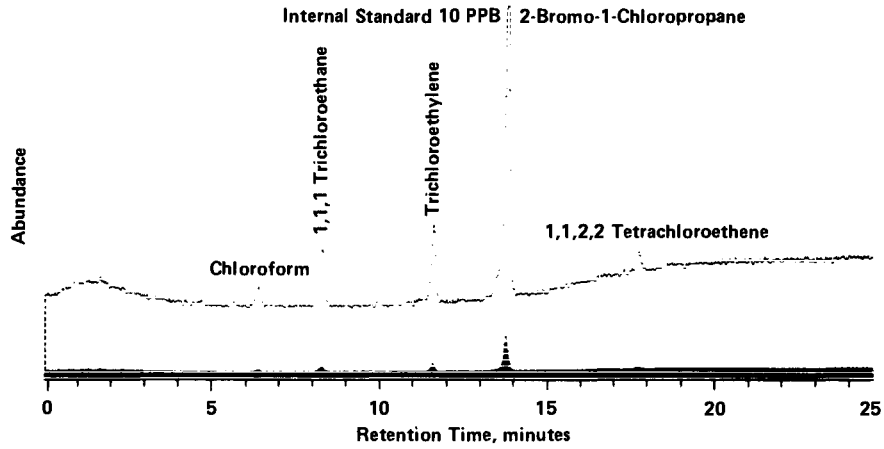

Fig. 1 Analysis of a water sample from San Francisco Bay

The relative retention time of the unknown compound is then compared with the relative retention time of the compound identified by the library search. The correlation of the retention times is combined with the library search correlation to produce a combined correlation factor which accurately defines the quality of the tentative identification of the unknown compound. If the total correlation factor is above 0.75, the identification can be considered positive.

The internal standards are very helpful in establishing accurate retention times. They also are used to accurately define a quantitative report. The total abundance peak heights for each component are compared to the peak height for the internal standard. A response factor table stored in the calculator memory converts these peak height ratios to actual concentration.

The response factor corrects for differences in purging efficiency and trapping efficiency. It also corrects for responses to the gas chromatograph and mass spectrometer. All of the qualitative and quantitative data is brought together and printed out by the calculator in a Final Report, Table 1.

The same analysis scheme is carried out when any of the other three types of analysis are requested. However, in these cases, the sampling is not controlled by the calculator. The operator must carry out the liquid/liquid extraction and subsequent concentration. The sample is then injected into the GC/MS by the operator. The GC/MS analysis and data reduction are then controlled and completed by the calculator. The Final Reports for these analyses have the same format as the report displayed in Table 1.

Peaks not identified as being in the library are labeled "UNKNOWN" and are given a tentative concentration level based upon a response factor of 1.00. These peaks can usually be identified through off-line library searches. Analysis of several different types of water samples have been successfully undertaken to demonstrate the versatility of this method.

TABLE 1 Final Report

Name	Spect. #	Ret. Time	Corr.	Conc.(ppb)
Chloroform	330	6.4	.984	0.27
1,1,1 Trichloroethane	332	8.2	.971	0.52
Trichloroethylene	336	11.6	.925	0.35
Internal Standard	340	13.8	.941	10.0
1,1,2,2 Tetrachloroethene	354	17.7	.912	0.55

In an effort to maximize reuse of reclaimed water, research is being conducted to improve advanced waste treatment facilities so that their effluent may be used as ancilliary water supplies. Water samples taken prior to and after teritiary treatment were analyzed to determine the efficiency of advanced waste treatment methods. (William Roman (1978)) Fig. 2 and Fig. 3 show the results. After secondary biological treatment and chlorination, there are high levels of chlorinated hydrocarbons. Almost all of these are eliminated or significantly reduced in the tertiary stages of treatment.

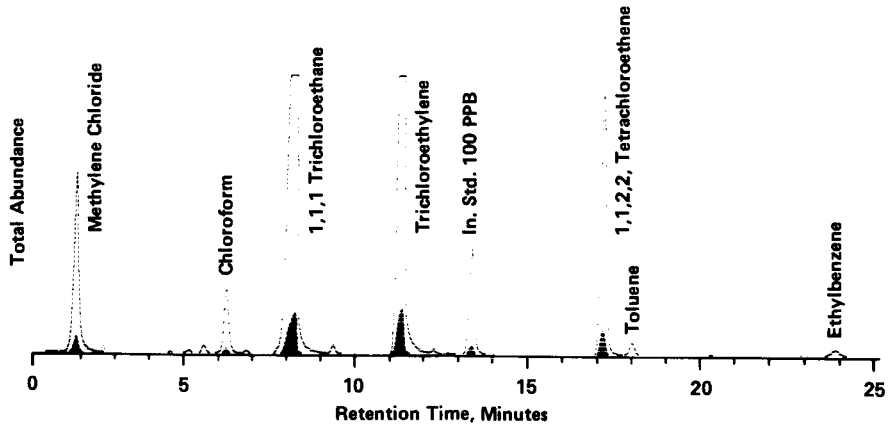

Fig. 2 Profile of sewage prior to tertiary treatment

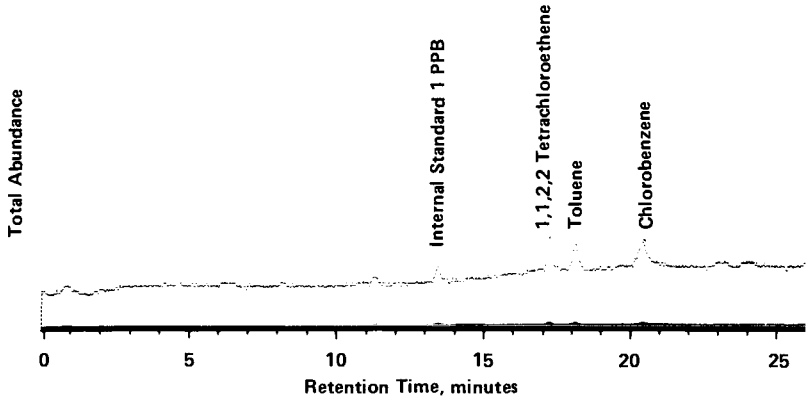

Fig. 3 Profile of sewage after tertiary treatment

Analysis of semi-volatile and nonvolatile components are carried out through the use of a liquid/liquid extraction procedure using methylene chloride (USEPA, 1977). Basic, neutral and acidic fractions are extracted. The basic and neutral fractions can be combined and analyzed together.

Samples "spiked" with known toxic compounds were analyzed via the base/neutral method. In each case, the system identified and properly performed a quantitative analysis for each GC peak observed. A representative chromatogram of a "spiked" sample is shown in Fig. 4.

The system has been used to analyze raw finished water samples for the presence of the consent decree compounds. Fig. 5 displays the chromatogram from a representative base/neutral extract. In this case, each of the several internal standards were identified (Peaks 1, 2, 3, 7, 8, 10) along with dibutylphthalate (Peak 9). The other peaks were not identified immediately but were defined as not belonging to the consent decree list.

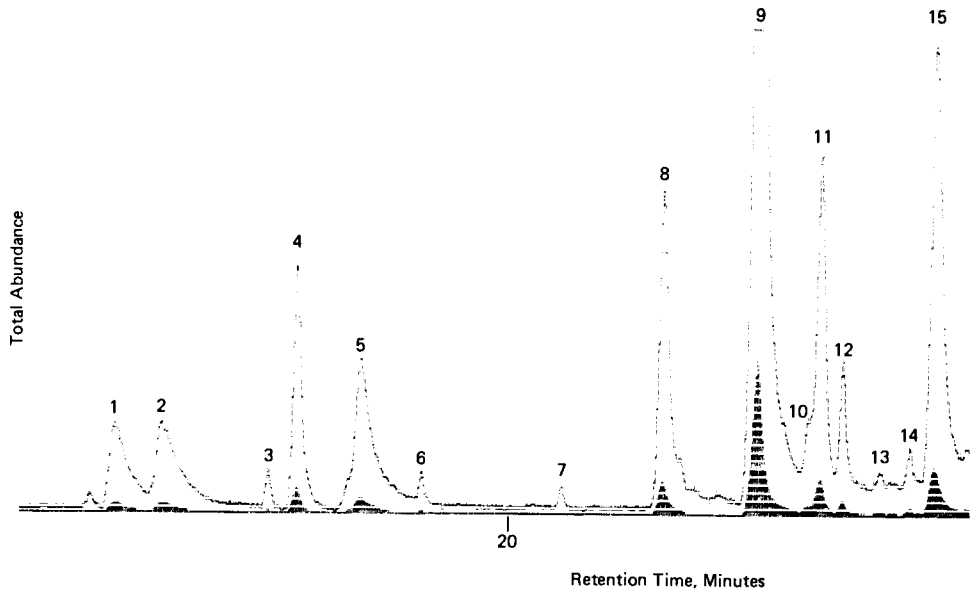

Fig. 4 Base/neutral extract standard

1.	1,3 Dichlorobenzene	8.	Acenaphthene
2.	1,2 Dichlorobenzene	9.	Diethylphthalate
3.	Septum Contaminant	10.	Septum Contaminant
4.	Hexachlorbutadiene	11.	Hexachlorobenzene
5.	Naphthalene	12-14.	Septum Contaminant
6.	Septum Contaminant	15.	Anthracene
7.	Septum Contaminant		

Figure 6 shows the analysis of an acid extraction from secondary treated sewage (William Roman (1978). This analysis resulted in the identification of the C_{30}, C_{32}, C_{34} and C_{36} normal alkanes. Quite surprisingly, a large concentration of bromocyclohexanol was detected. This alerted the sewage disposal plant to an unusual source of discharge of this compound, and presented further contamination by that source.

In each analysis, the system automatically recorded the spectra, corrected the spectra for background contamination, identified the unknown compounds via spectral library searches, confirmed the identification via relative retention time correlations, performed a quantitative analysis and printed out a complete qualitative and quantitative report. Unfortunately, space limitations do not allow reproduction of these final reports in this publication.

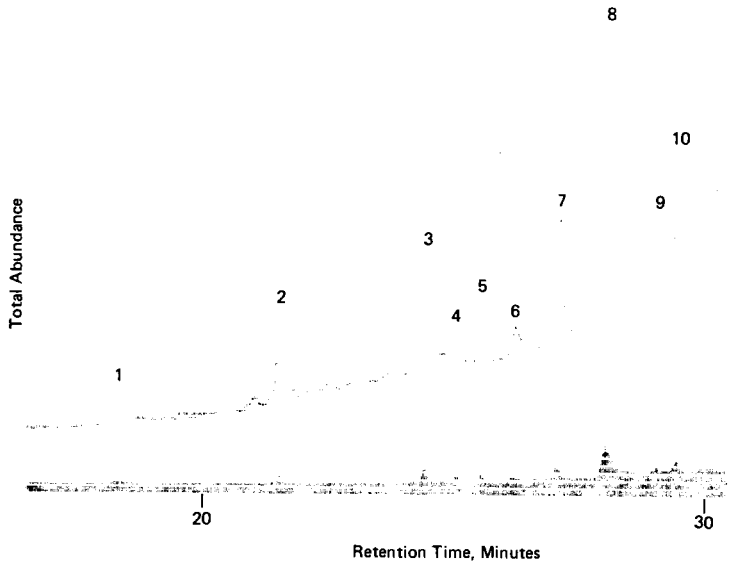

Fig. 5 Base/neutral extract of raw finished water

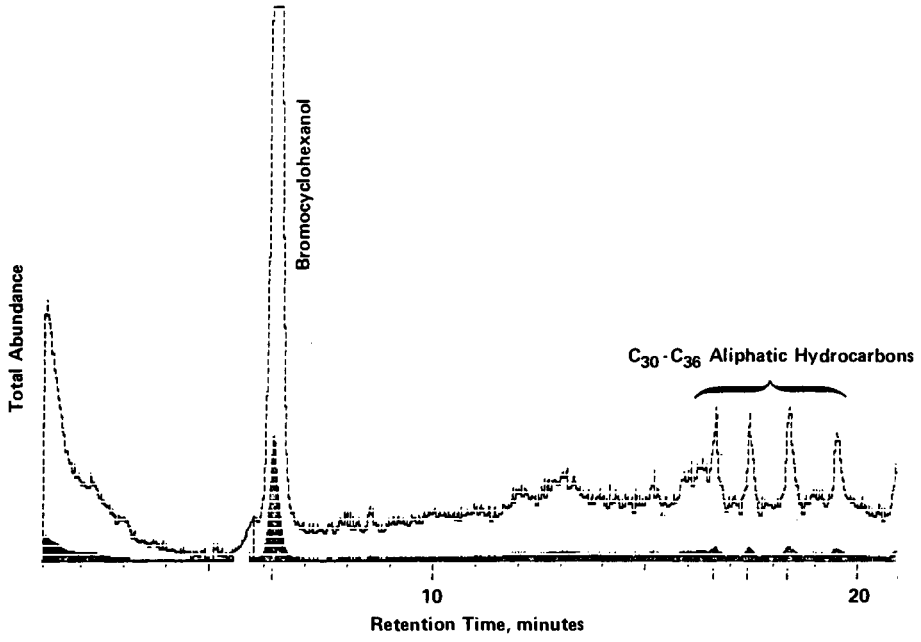

Fig. 6 Acid extract from secondary treated sewage

CONCLUSION

These examples demonstrate the utility of a GC/MS system in the routine analysis of organic constituents in water. Positive identification is achieved through the use of a fast accurate library search routine. Additional confirmation can be achieved by comparison of relative retention times. Accurate quantitation results from the ability to compare responses of contaminants to the response of an internal standard. The high sensitivity of this technique enables positive identification of compounds at levels below 1 ppb.

REFERENCES

U.S. EPA (1977), Protocol for Sampling and Analysis Procedures for Survey of Industrial Effluents for Priority Pollutants, Environmental Monitoring and Support Laboratory, Cincinnati. Note: A few of these compounds are actually technical mixtures such as chlordane and PCB 1242.

Bellar, T. A., and Lichtenberg, J. J., (1974), Determining Volatile Organics at Microgram-per-Litre Levels by Gas Chromatography, Am. Water Works, J., 66, 739.

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Trace Analysis of Organics in Water by Gas Chromatography

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ABSTRACT

This paper discusses column technology required for separation of 114 organic water pollutants proposed for investigation by America's Environmental Protection Agency. The procedure outlined by the EPA is to separate pollutants into five categories: volatiles, semi-volatiles, basic, acidic and pesticides, each requiring a specific extraction technique and column technology.

Volatiles found in water consist of halogenated hydrocarbons and light aromatics which are analyzed using a Carbopack C/0.2% Carbowax 1500, 8 foot x 1/8 inch column. An improvement is observed when Carbopack B/1% SP-1000 is used.

Semi-volatiles and pesticides are extracted from water with methylene chloride, distilled in glass, and separated on a 1% SP-2250 column.

Acidic compounds consist mainly of the chloro- and nitro- phenols, which are proposed to be analyzed on a Tenax column. This column was found to have some limitations in analysis of ppm concentration of nitrophenols. A new column packing, 1% SP-1240-DA, was developed and proven able to analyze all the phenols at the ppm concentration.

In conclusion, analysis of water for organic pollutants is conducted with only three columns. With use of specific detectors, chromatographic columns tailored for specific organic pollutants allows use of gas chromatography for complete screening of water samples, followed by mass spectrometry confirmation.

INTRODUCTION

In 1974, the Congress of the United States enacted into law the Safe Drinking Water Act, requiring the Environmental Protection Agency (EPA) to establish methods for control and analysis of toxic substances in waste water. As a result of this law, the EPA has issued a protocol outlining the analytical methods for the determination of 129 pollutants. The procedures were based

on the best approach to the analysis of pollutants using conventional chromatographic techniques and instrumentation. This paper outlines and discusses the column technology proposed by EPA, as well as improvements to the analysis of those trace organic pollutants in water.

Of the 129 pollutants listed in the protocol, 114 are organic compounds which can be extracted from water and analyzed by gas chromatography. Concentration of the organics found in water is required because most organics are at nanogram per milliliter or lower concentration. The methods of concentration normally used are liquid-liquid extraction for non-volatiles, and purge-trap techniques for volatiles. One liter of water is normally extracted for non-volatiles, and 20 milliliter for volatiles. The non-volatile organics are divided into specific groups by sequential extraction at neutral, alkaline, and acidic pH's. As a result, the 114 organics can be divided into four categories: volatiles, pesticides and PCB's, base neutrals, and acidic compounds.

VOLATILES IN WATER

The volatile organics are compounds which are easily vaporized and removed from water by displacement with helium gas. After the volatiles are purged from the water sample, they are collected on a solid adsorbent and the helium vented. The purge-trap technique recommended is the same as described by Bellar and Lichtenberg (3).

The adsorbent proposed is Tenax-Silica Gel 15, a combination chosen to collect heavy vs. light molecular weight volatiles, respectively. After the volatiles are collected, they are rapidly heat-desorbed onto the chromatographic column.

The procedure specified by EPA for the analysis of the volatile organics proposes the use of an 8 ft. x 1/8 inch SS column with 80/100 mesh Carbopack C, modified with 0.2% Carbowax 1500. The separation of the 30 volatiles is obtained by decreasing volatility, which is inversely related to the degree of halogenated substitution. Figure 1 shows the analysis of the volatiles minus the five gaseous volatiles and aromatic compounds; benzene, toluene, and ethyl benzene. Those volatiles of particular interest are chloroform, bromodichloromethane, chlorodibromomethane and bromoform, all byproducts of the chlorination of drinking water. The analysis of the volatiles shown here were conducted with a Hall conductivity detector adapted to a Varian 3700 chromatograph.

Acrolein and acrylonitrile are also considered as volatiles, but they are not efficiently recovered in the purge and trap procedure. Therefore, they are analyzed by direct aqueous injection on the Carbopack C/0.2% Carbowax 1500 column. The lowest detectable amount of acrolein and acrylonitrile reported by EPA is 0.1 ng/$\mu\ell$, when analyzed by direct aqueous injection using a flame ionization detector.

Detection of specific halogenated volatiles is conducted with either a Hall conductivity, microcoulometric, or electron capture detector. The use of these detectors is recommended over other types of GC detectors because of selectivity for halogenated compounds and increased sensitivity. Those aromatics listed as volatiles do not respond to the halospecific detector mentioned previously; they require the use of a flame ionization or

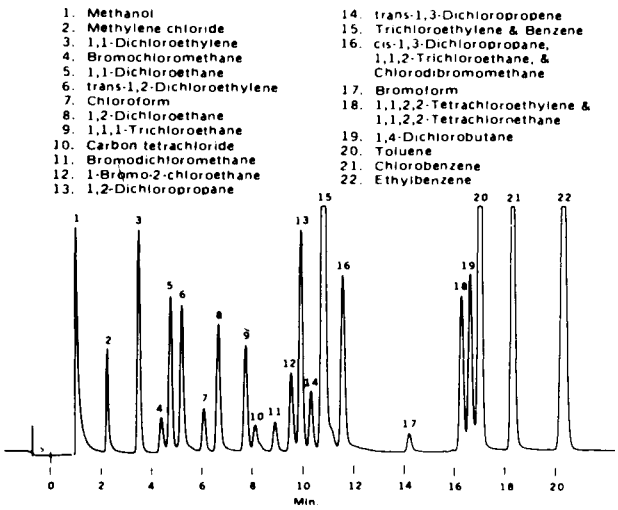

Fig. 1 Analysis of Volatile Pollutants

80/100 Carbopack C/0.2% Carbowax 1500, 6 ft. x 1/8 in. SS, Column temp. – 3 min: @ 60°C, 8°C/min. to 160°C, Flow rate – 30ml/min. N_2, Detector – FID; 64 x 10^{-11} AFS, Sample size – 0.5µl of 1mg/cc synthetic mixture in methanol, Instrument – Varian 3700

photoionization detector.

To overcome the upper temperature limit of 175°C for Carbowax 1500, and the limited separation of the light volatile gases, a new column packing of Carbopack B with 1% SP-1000, 60/80 mesh is proposed. This column packing was previously described by Bruner, Bertoni, and Crescentini (4) at the 12th International Symposium on Chromatography in Baden-Baden in September of 1978.

With a higher maximum temperature of 225°C and the increased surface area and liquid loading, both problems are overcome as shown in Figure 2. Note the resolution of the light gases, peak 1-4; but without the loss of resolution of the late eluting volatile organics. The analysis of the volatiles shown in Figure 2 was conducted with a flame ionization detector to show the elution order of the aromatic compounds relative to the halogenated volatiles. The analysis of the volatiles shown in Figs. 1 and 2 were by direct syringe injection of dilute standard solutions in methanol. Figure 3 is representative of the analysis of volatiles in a water sample at nanogram per microliter concentrations. The chromatogram shown was obtained from a 10ml waste water sample, compliments of the Analytical Service Section, the Surveillance and Analysis Division, of Region IV Environmental Protection Agency in Athens Georgia, U.S.A. The chromatogram is a reproduction of a mass spectrometry analysis using Carbopack A with 0.4% Carbowax 1500. Carbopack A/0.4% Carbowax 1500 is no longer available, and has been replaced by Carbopack C/0.2% Carbowax 1500.

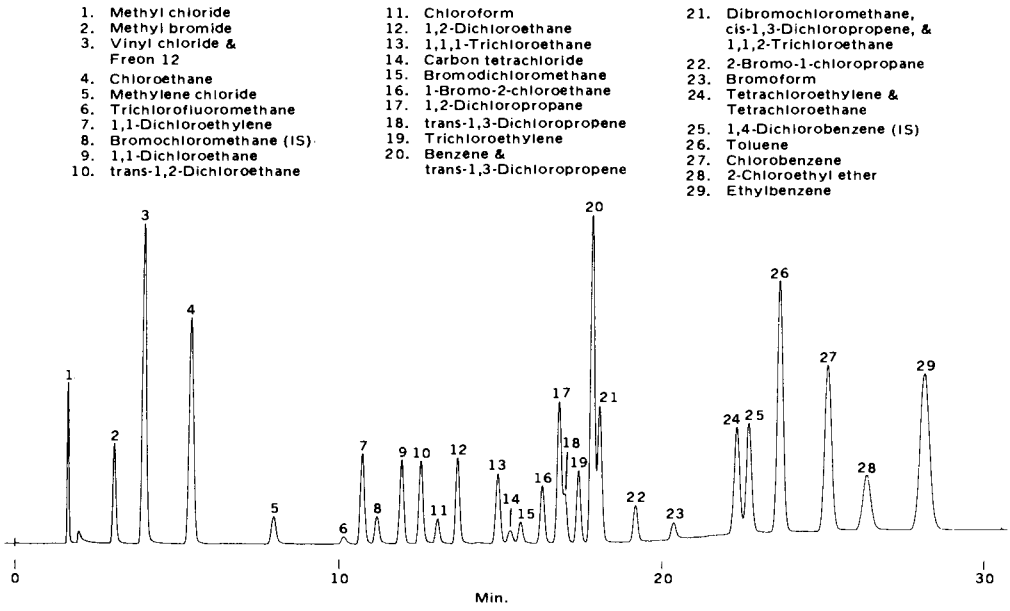

Fig. 2 Analysis of Volatile Pollutants

60/80 Carbopack B/1% SP-1000, 10 ft. x 2mm ID glass, Column temp. – 4 min. hold, 50°C to 200°C @ 10°C/min., Flow rate – 30ml/min. N_2, Detector – FID; 16 x 10^{-11} AFS, Sample – synthetic mixture, 0.5mg/ml volatiles in dodecane

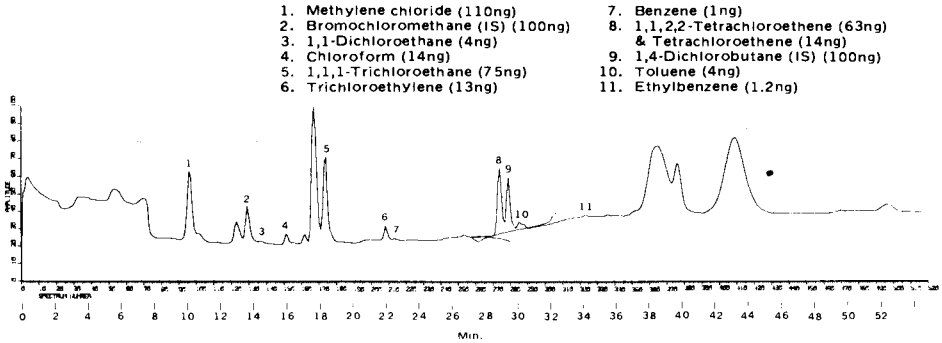

Fig. 3 Analysis of Waste Water for Volatile Pollutants

60/80 Carbopack A/0.4% Carbowax 1500, 10 ft. x 2mm ID glass, Column temp. – 50°C to180°C @ 8°C/min., Flow rate – 20ml/min. He, Detector – Finnigan Mass Spec., Sample – Water sample

Compliments of Analytical Services Section, Surveillance and Analysis Division, Region IV, U.S. Environmental Protection Agency, Athens, GA.

PESTICIDES - PCB'S

In the analysis of the pesticides, PCB's, and base neutrals a single multi-purpose column, 1% SP-2250 on 100/120 Supelcoport was chosen. SP-2250 is a methyl-phenyl silicone (50/50) liquid phase with a temperature range of 50° to 360°C, which allows for the complete analysis of the afore mentioned compounds. The distinction in the analysis of the respective class of compounds is made in the extraction technique used.

The pesticides and PCB's are extracted from water with 15% methylene chloride in hexane, concentrated to 10 milliliter volume, and taken up in hexane before the final concentration to 1ml volume (1). Methylene chloride distilled in glass, or of comparative purity, is the only recommended grade to be used for extraction. Figure 4 shows the analysis of the pesticides listed in the protocol using a 6 ft. x 2mm ID glass column containing 1% SP-2250 on 100/120 Supelcoport. Though the resolution of the pesticides is limited with this column, the pesticides are differentiated from PCB's, Chlordane, and Toxaphene which show multiple isomer peaks and elute after the pesticides. The analysis shown here was conducted on a Hall detector which has a minimum detection limit of only a nanogram/microliter for most of the pesticides due to the high background noise. As an alternative to the 1% SP-2250 packing, the mixed phase 1.95% SP-2401/1.5% SP-2250 on 100/120 Supelcoport would be an excellent choice. Figure 5 shows the analysis of 14 pesticides listed in the protocol using this mixed phase packing. Chlordane and toxaphene elute after the pesticides. The analysis shown here was conducted with a pulse frequency electron capture detector with detection limits of 5 picograms/microliter for most of the pesticides. Since the PCB's would overlap the pesticides with this packing, sample clean-up with silica gel or Florisil is required to remove the PCB's (2).

BASE NEUTRALS

The base neutrals include a broad range of organic compounds such as chloro-ethers, nitrous amines, chloro- and nitro- aromatics, and polycyclic aromatics. In the analysis of base neutrals, the water sample is treated with 6N-NaOH to achieve a pH higher than 11, then extracted with methylene chloride. The extract is analyzed on the same column used in the pesticide analysis, the 1% SP-2250.

Heavy tars and oils will occasionally be present in water samples and interfere in the analysis by not coming off the column. With the 360°C limit of SP-2250, they are easily burned from the column by raising the column temperature after the sample analysis is completed. However, because of the adsorption and tailing of many base neutral compounds, an alternate column, 3% SP-2250-DB, is proposed. The DB designation means specially deactivated for basic compounds. Figure 6 shows the base neutrals analysis with the 3% SP-2250-DB column. Noteworthy in this figure is the improved peak shape on the early eluting base neutrals, but also note the 275°C temperature limit of 3% SP-2250-DB to the addition of the DB deactivation.

ACIDIC COMPOUNDS - PHENOLS

The last group of pollutants to be discussed are the acidic compounds, consisting of 11 substituted phenols. Prior to the extraction of the water

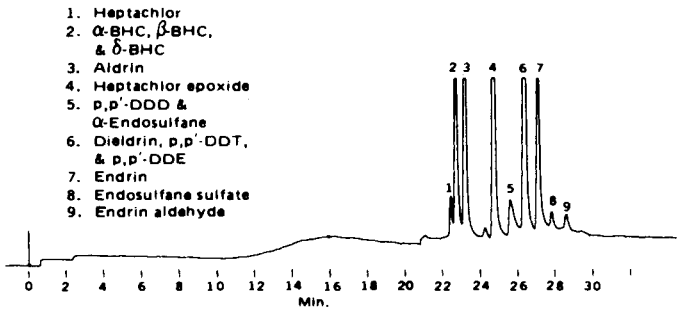

Fig. 4 Analysis of Pesticides on 1% SP-2250

1% SP-2250 on 100/120 Supelcoport, 6 ft. x 2mm ID glass, Column temp. – 4 min. @ 50°C, 8°C/min. to 260°C, Flow rate – 30ml/min. N_2, Detector – Hall Conductivity; 10 x 4, Sample size – 0.1μl of 1ng/μl pesticides in isooctane, Instrument – Varian 3700

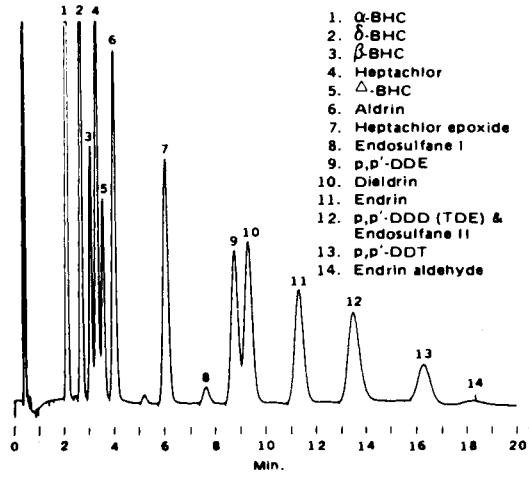

Fig. 5 Analysis of Pesticides on 1.5% SP-2250/1.95% SP-2401

1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport, 6 ft. x 4mm ID glass, Column temp. – 200°C, Flow rate – 70ml/min. N_2, Detector – EC Ni_{63}, Sample size – 0.5μl of 1ng/μl of pesticides in isooctane, Instrument – HP-5840

sample with distilled-in-glass methylene chloride, the water sample is acidified, using 6N-HCl, to a pH of 2. The protocol proposes the use of a Tenax 6 ft. x 2mm ID glass column for the analysis of phenols.

For this analysis, Tenax is marginal at best because of tailing and adsorption of many of the nitro phenols. Shown in Figure 7 is the analysis of a synthetic standard at 50 nanogram per microliter with a total loss of 2,4-dinitro phenol, and tailing of the pentachloro phenol and 4-nitro phenol peaks.

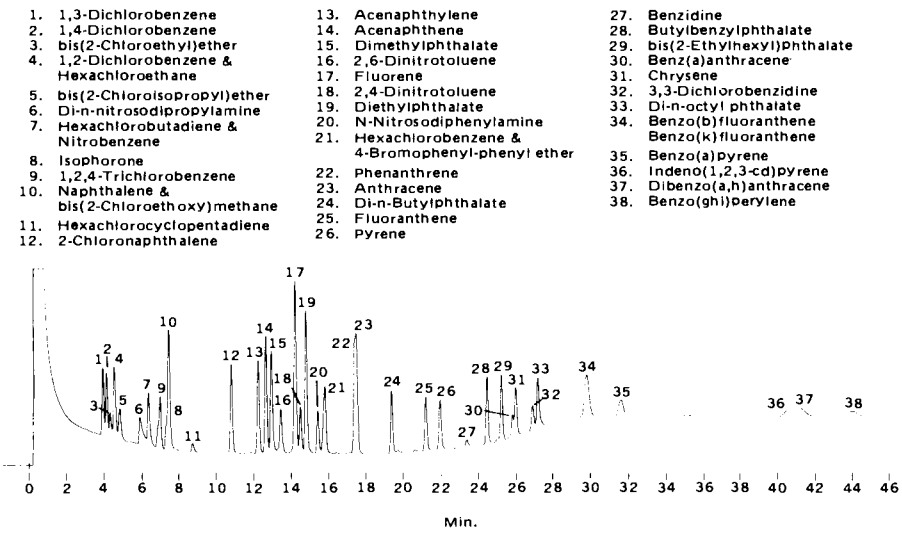

Fig. 6 Analysis of Base Neutrals on 3% SP-2250-DB

3% SP-2250-DB on 100/120 Supelcoport, 6 ft. x 2mm ID glass, Column temp. – 4 min. @ 50°C, 8°C/min. to 260°C, Flow rate – 20ml/min. N_2, Detector – FID; 16 x 10^{-11} AFS, Sample size – 1μl of 0.05mg/ml standard in methylene chloride, Instrument – Varian 3700

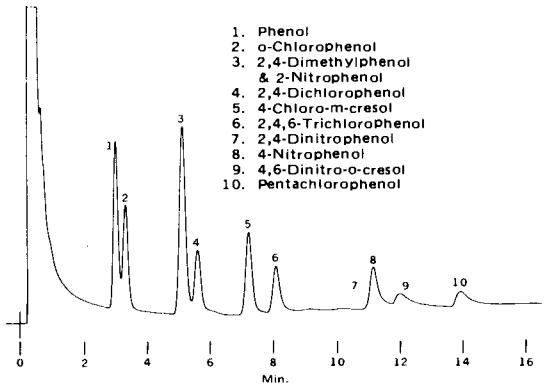

Fig. 7 Analysis of Phenols on Tenax

Tenax, 60/80, 6 ft. x 2mm ID glass, Column temp. – 180°C to 300°C @ 8°C/min., Flow rate – 30ml/min. N_2, Detector – FID; 8 x 10^{-11} AFS, Sample size – 1μl of 0.05mg/ml phenol standard in methanol, Instrument – Varian 3700

Supelco, working in conjunction with the EPA, has developed a new chromatographic packing, SP-1240-DA, specially deactivated for the analysis of acidic compounds. The analysis of a synthetic phenol standard using this packing is shown in Figure 8.

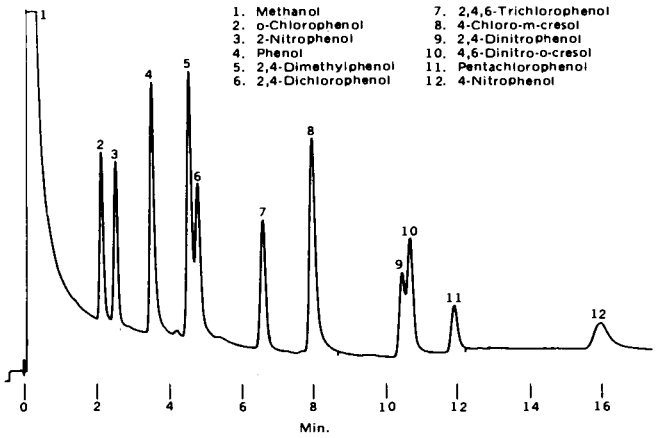

Fig. 8 Analysis of Phenols on SP-1240-DA

1% SP-1240-DA on 100/120 Supelcoport, 3 ft. x 2mm ID glass, Column temp. – 85°C to 190°C @ 8°C/min., Flow rate – 30ml/min. N_2, Detector – FID; 16 x 10^{-12}, Sample size – 1µl of 0.05mg/ml phenol standard in methanol, Instrument – HP-5710

Noteworthy in this chromatogram is the lack of tailing peaks and the resolution of the dinitrophenols, pentachlorophenol and p-nitrophenol.

With an upper temperature limit of 200°C, SP-1240-DA is not recommended to be used to 200°C for long periods of time. Though bleed will occur at 200°C, no interferences occur in mass spectrometry analysis.

Figure 9 shows a reconstructed chromatogram from the mass spectrometry analysis of a water sample, conducted by the Analytical Services Section at the EPA in Athens, Georgia. Notice that nanogram per microliter analysis of phenols has been obtained here. The unlabeled peaks shown in this figure are not phenolic in nature, therefore, no further attempt was made to identify them. During the EPA evaluation of SP-1240-DA, it was necessary to periodically replace the phosphoric treated glass wool at the column inlet. This prevented adsorption of the phenols after the glass wool had accumulated oils and other deposits from the sample injection. Using an automatic sampler for unattended overnight analysis, the SP-1240-DA column was used continually for a period of over two months with only periodic changing of the glass wool at the inlet to the column.

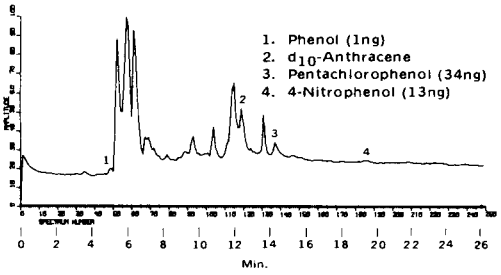

Fig. 9 Analysis of Waste Water Sample for Phenols

1% SP-1240-DA on 100/120 Supelcoport, 3 ft. x 2mm ID glass, Column temp. – 50°C to 170°C @ 6°C/min., Flow rate – 20ml/min. He, Detector – Finnigan Mass Spec., Sample – Water sample

Compliments of Analytical Services Section, Surveillance and Analysis Division, Region IV, U.S. Environmental Protection Agency, Athens, GA.

With the current interest in the use of nickel tubing vs. glass tubing, the analyses of phenols with SP-1240-DA packing in nickel tubing was investigated. The only difference seen was the slight improvement in resolution with glass, as expected. A side note in the use of nickel tubing was the slow conditioning of the metal column, 2°C/min. from ambient to 200°C, which may further enhance deactivation of the tubing by coating the tubing walls during conditioning. This phenomenon is being investigated with other types of compounds and will be published at a later date.

SUMMARY

In conclusion, an improvement has been shown in the analysis of the volatiles in water by the use of Carbopack B with 1% SP-1000. A 3% SP-2250-DB has been shown to improve the analysis of the base neutrals by decreasing adsorption with deactivation of the support. The development and intensive evaluation of SP-1240-DA provides a welcomed and proven column packing for the analysis of phenols in water.

I would like to acknowledge the help of the EPA, Region IV in Athens, Georgia in the evaluation of both the SP-1240-DA and 3% SP-2250-DB column packings.

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(4) Bruner, F., Bertoni, G., and Crescentini, G. (Sept. 1978) Critical Evaluation of Sampling and Gas Chromatographic Analysis of Halocarbons and Other Organic Air Pollutants. Presented at The 12th International Symposium on Chromatography at Baden-Baden.

Analysis of Volatile Components in Waste Water from a Urea-formaldehyde Glue Plant

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ABSTRACT

A simple analytical method using gas-chromatography is described in order to determine the formaldehyde,methylal,methanol,and methyl formate contents in waste water from a urea-formaldehyde glue plant using Chromosorb 101 and Ethofat 60/25 as stationary phase.The optimum amount of stationary phase is chosen from the study of the resolution of each pair of components respect to the phase percentage.Preconcentration techniques in cases of very diluted solutions are described,and it's shown that the fractional distillation is the most adequate in such cases.

KEYWORDS

Gas-chromatography,formaldehyde,preconcentration,urea-formaldehyde resins,waste-water volatiles.

INTRODUCTION

In this work,a simple analytical method using gas-chromatography is described,in order to determine the formaldehyde,methylal,methanol,and methyl formate contents in waste water from a urea-formaldehyde glue plant.Glue can be manufactured by two different procedures,depending on the initial formaldehyde concentration of the solution used.In the conventional process,aqueous solutions of formaldehyde with a concentration between 30-40% are used,producing waste water with a 0.5 to 2% formaldehyde concentration as well as other components with a concentration between 0.1 and 1%. In the other process,developped and patented in several countries

by Derivados Forestales S.A.,formaldehyde of 68-70% is directly obtained from the product manufacturing plant,thus avoiding the emission of effluents.In figure 1,a diagram of these processes can be seen.

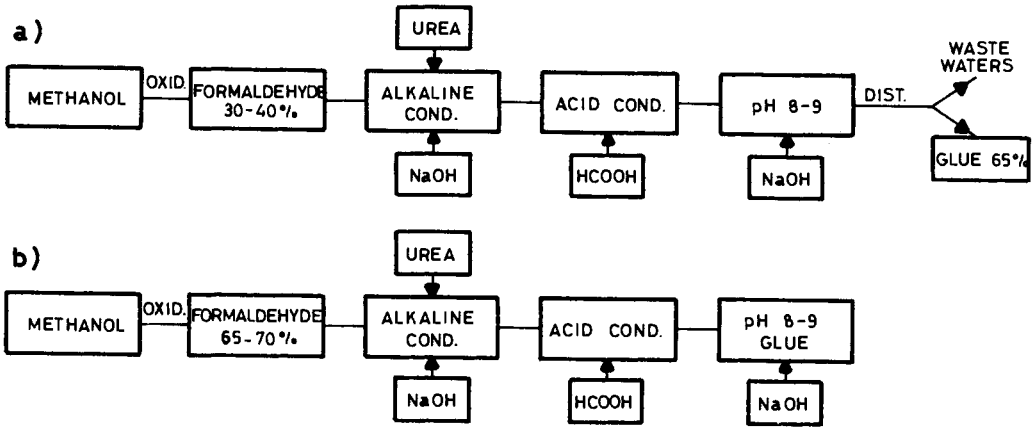

Fig. 1. Diagrams of the two different procedures of manufacturing glues: a) conventional process,b) Derivados Forestales S.A. process.

Although in D.F.'s process no distillation step exists,there is always some slightly pollution on waste water,produced by the cleaning of the reactors and the floors;they must be under control.Thus,there are several chemical as well as instrumental methods in order to determine the pollutants in such waste water. This study is limited to the aforementioned volatile substances, which may help to show the degree of pollution,the index of the condensation course,and the conventional process yield.

To determine every substance in one sample takes quite a long time.Undoubtedly gas-chromatography is the quickest method,but up to now two different stationary phases have been required,in order to make a full analysis of the aforementioned substances.Up till now,the most commonly stationary phases used were Ethofat 60/25(polyethylenglicol monostearate) (Bombaugh and Bull,1962;Jones,1967) or other stearates on Teflon 6,Columpak T,and Polykhrom (Mann and Hahn,1967;Paronyan and Sarkisyan,1974;Stevens and Percival,1964;Styskin,1973,1974),or on diatomeaceous earth (Bozesanu, 1973;Epimakhov and Manchevskaya,1976;Sidorov and Khvostikova,1968) and those using an porous polymer adsorbent such as the Porapak Q, N, Chromosorb 101,102, and Polysorb-1 (Krasirov,Rastunov,and Nakhutin,1975;Onuska,and co-workers,1969;Otvos,and co-workers,1971; Shushunova,Gudovicheva,and Schernichova,1974;Tkacheva,and co-workers,1977).Although each one of them may be applicable to special

problems,such as the determination of formaldehyde in aqueous solutions,or formaldehyde in methanol,they cannot efficiently separate the five components of the mixture,including water,and are even less efficient when some of the components have a high enough concentration to disturb the proper separation of the rest.Thus, the Ethofat phase,or one of a similar composition,is very suitable for determining the formaldehyde content,as the later is retarded with respect to the other components of the mixture.However,it does not allow a proper separation of the rest of the components.On the other hand,the columns filled with Chromosorb 101 (or similar) properly separate every component of the mixture,except the formaldehyde.The mechanism by which the two phases act are completly different,one by partition and another by adsorption, hence it was thought that adding some given quantity of Ethofat to the Chromosorb 101,the two advantageous effects might be combined,so that,with its long retention time,formaldehyde would not interfere and at the same time the correct separation of the rest of the components would be accomplished.

RESULTS

In order to know the optimum percentage of Ethofat to be added to the Chromosorb 101,several columns were prepared having between 0,3,10,15,20,25,and 30% of Ethofat 60/25 respectively on the active suport,and,afterwards,mixtures of the five components in different proportions(table 1) were chromatographed on these columns at the experimental conditions showed in table 2,in order to know their effect on the separation as well.

TABLE 1 Samples Tested

Sample	Methanol %	Formaldehyde %	MethylFormate %	Methylal %	Water %
B	70.0	6.0	5.0	5.0	14.0
C	19.4	4.2	5.5	5.5	65.4
D	34.3	10.3	6.3	6.3	42.8
E	86.7	0.0	0.0	0.9	12.4
F	88.4	0.0	0.0	10.5	1.1
G	86.0	0.0	0.0	0.1	13.9
H	25.0	0.0	0.0	74.4	0.6
I	0.2	0.3	0.1	0.1	99.3
J	0.007	0.010	0.006	0.006	99.9

In figure 2,Log V'_R is represented with respect to the percentage of Ethofat,and in table 3 the corresponding values are shown.Table 3 shows that the Ethofat effects the different components in different way,and the degree of separation is directly proportio-

nal to the polarity.Thus,for very polar components,the effect of increasing the quantity of stationary phase is of the same sign, that is to say,the retention time slowly increases with the quantity.Thus,for those less polar components,methylal and methyl formate,the effect is irregular.The results obtained show the performance of two different forces,that of the support and that of the liquid phase.

TABLE 2 Operating Conditions

This work was done with a Perkin-Elmer Model 990 gas chromatograph equipped with a thermal conductivity detector.
Columns : L= 2 m, and $\emptyset_i$ = 2 mm

		Samples B-H	Samples I,J
Temperature:	Injector	200ºC	200ºC
:	Detector	200ºC	200ºC
:	Column		
	Initial	70ºC(10min)	60ºC(10min)
	Program rate	24ºC/min	16ºC/min
	Final	105ºC(8min)	105ºC(8min)
Sample size:		0.2 ul	5.0 ul
Bridge current:		175 ma.	275 ma.
Carrier gas:		Helium	Helium
Flow rate :		30 ml/min	30 ml/min

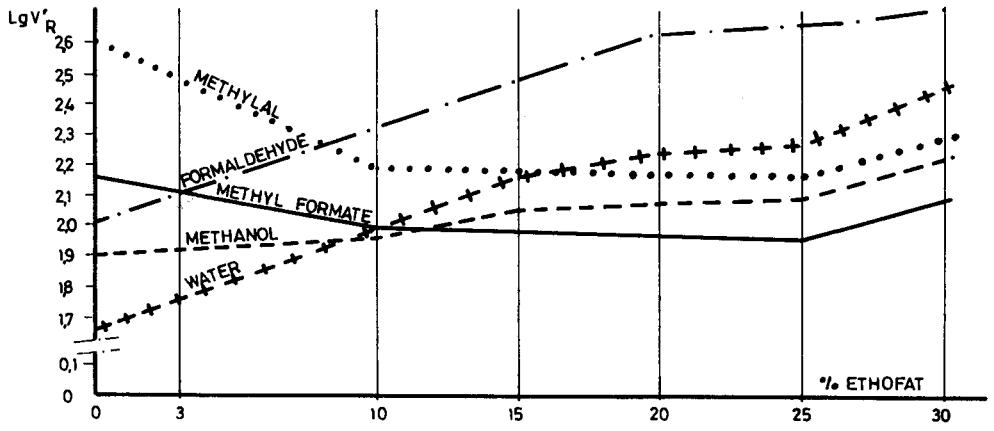

Fig. 2. Dependence of the Log V'_R respect to the percentage of Ethofat.

TABLE 3 Values of the Log V'_R

Columns: %Ethofat on Chromosorb 101	0	3	10	15	20	25	30
Methanol	1.90	1.92	1.96	2.07	2.12	2.14	2.28
Water	1.65	1.85	2.00	2.17	2.27	2.30	2.49
Methylal	2.60	2.40	2.23	2.22	2.21	2.19	2.33
Methyl Formate	2.15	2.10	2.01	2.00	2.00	2.00	2.14
Formaldehyde	2.00	2.09	2.33	2.48	2.64	2.68	2.76

We also see that,from a given phase percentage on the adsorbent (25%),the five components have a similar behaviour,which could mean that in this percentage the phase effect takes advantage on that of the support.However,the resolution is not given by the values obtained previously.The resolution can be seen in figure 3, in which R has been calculated according to the expression:

$$R= 2(d_{r1}- d_{r2})/(W_1 + W_2)$$

for each two components of the mixture with respect to the percentage of the stationary phase.

From this expression,it can be inferred that the higher point of the resolution minimum is found at Ethofat values between 20 and 25 %,and has an approximately value of 2,which is sufficient for a good separation of the mixture in most cases.If we assume a linear variation of R between these two points,the ideal column, which would satisfy both criteria previously mentioned,would be of 22% of Ethofat 60/25 on Chromosorb 101.This column was prepared and experimentally tested.In figure 4 the chromatograms of solution D show that the mixture is correctly separated into its components using a 22% Ethofat cclumn as compared to the 20 and 25% columns.

Afterwards,several diluted aqueous solutions of the mixture were chromatographed(figure 5).Having established the correct column conditions,it was found that the minimum determinable quantity of formaldehyde was about 30 ppm,as it appears at the tail of the water peak,and that the separation is not perfect.The other components do not present any difficulty,and lower concentrations can be determined.

By using this method,the solutions of waste water from standard glue manufactures,cointaining significant quantities of formal-

dehyde,can be easily analysed,and the four volatile components can be determined in only one analysis.

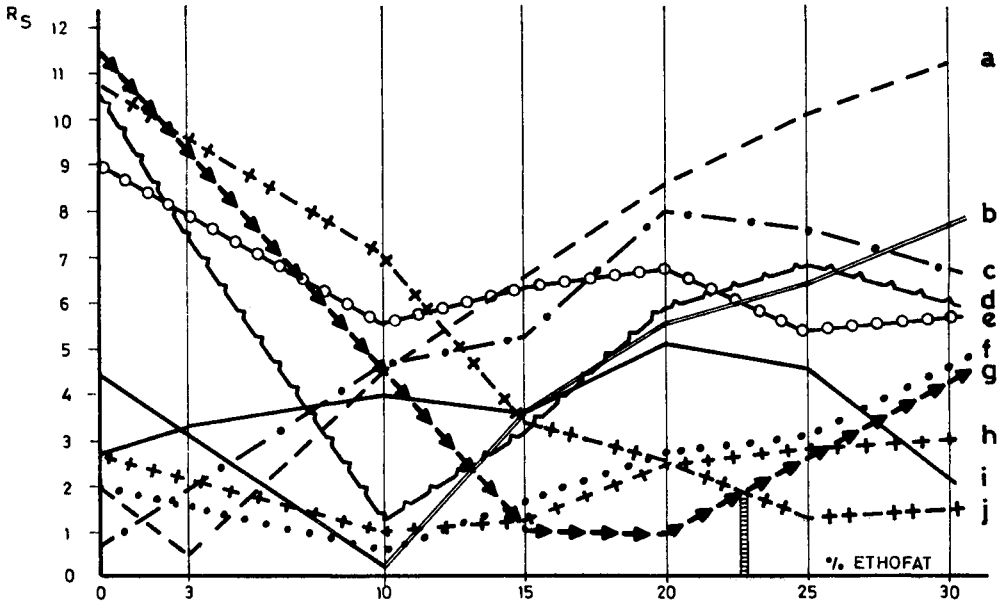

Fig. 3. Representation of the resolution for each two components of the mixture: a) Methyl Formate-Formaldehyde, b) Methyl Formate-Water, c) Methanol-Formaldehyde, d) Methylal-Formaldehyde, e) Methylal-Methyl Formate, f) Methanol-Water, g) Methylal-Water, h) Methanol-Methyl Formate, i) Water-Formaldehyde, j) Methanol-Methylal.

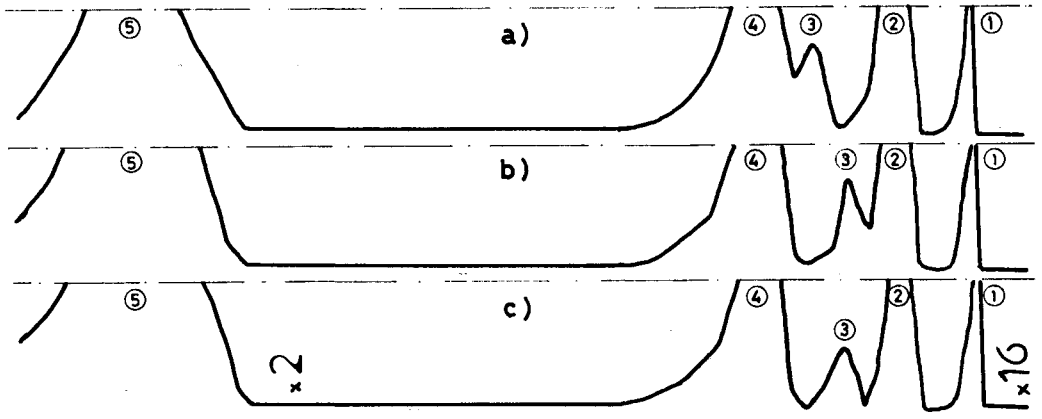

Fig. 4. Chromatograms of sample D(operating con-

ditions in table 2) : 1) Methyl Formate, 2) Methanol, 3) Methylal, 4) Water, 5) Formaldehyde.
Columns of : a) 20% Ethofat on Chromosorb 101, b) 25% Ethofat on Chromosorb 101, c) 22% Ethofat on Chromosorb 101.

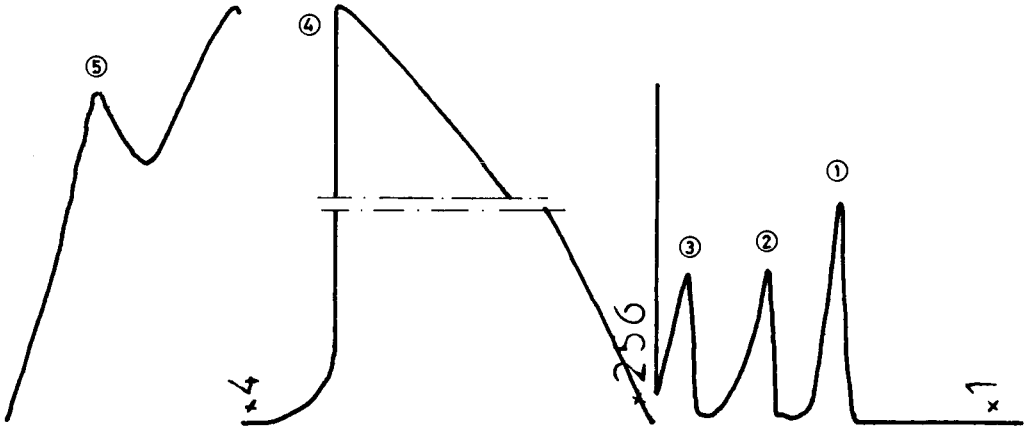

Fig. 5. Chromatogram of sample J(operating conditions in table 2) : 1) Methyl Formate, 2) Methanol, 3) Methylal, 4) Water, 5) Formaldehyde.Column of 22% of Ethofat on Chromosorb 101.

Since the waste water from cleaning invariably contains a formaldehyde concentration lower than the detectable 30 ppm,a preconcentration is necessary.Of the several procedures tested in table 4, only fractional distillation was effective.

TABLE 4 Methods of Preconcentration

Fractional Distillation
Adsorbents:
Active charcoal
Alumina
Silica gel
Organic polymers:Amberlite XAD
Ion exchange resins:Amberlite IRA-400 OH polystyrene matrix, Amberlite IRA-45 OH polystyrene matrix, Interanión MG OH acrylic matrix,Amberlite IRA-458 OH acrylic matrix

Although some tested type of active charcoal retains the formaldehyde,it does so slowly that is not analytically useful.In the cases of the ion exchange resins,only the OH form of weak-anionic ones retains the formaldehyde,provided its matrix is acrylic,as the polystyrene ones are not effective.However,the elution must be effected with NaOH 2 N,and the eluent cannot be directly injected into the chromatograph.Furthermore,it does not retain the other components.Only the distillation method allows the concentration of them. Using this procedure,solutions with quantities up to 1-5 ppm were analyzed.

However,as formaldehyde is very soluble in water owing to the formation of methylenglycols,when these diluted solutions are distilled an equilibrium is established,and the formaldehyde is not eliminated during the first fractions,but appears in all of them.It is known that the elimination of the formaldehyde from these diluted solutions is affected by pressure,which increases the balloon temperature,so displacing the equilibrium to form monomer formaldehyde.The same effect can be achieved by adding some very soluble salt,such as calcium chloride or sodium sulfate,in quantities near to the saturation point.Distillation recovers 90 % of the formaldehyde,into a volume 5% of the original;is a 20 fold concentration. The error margin is about 10%,which in view of the small quantities is not significant.In table 5 the results obtained are shown.

TABLE 5 Fractional Distillation

Working conditions:	
Column length 1.20 m with multifil wire packing	
Column stillhead with dephlegmator and total reflux during 1h 30 min	
Amount of sample to distil	: 3000 ml
Amount of distillate	: 150 ml
Recovery of formaldehyde without Na_2SO_4	: 45 %
Recovery of formaldehyde with Na_2SO_4	: 90 %
Amount of Na_2SO_4 added to the distillation flask	: 40 %
Distillation flask temperature(Na_2SO_4)	: 108 ºC

In order to achieve a better separation between water and formaldehyde,allowing the direct determination of lower quantities of formaldehyde,the same tests,with other adsorbents of the porous polymer type(Porapak and the rest of the Chromosorb century series) are being carried out.Similarly,the effects that the support and the stationary phase have in the behaviour of several components of different polarity are being studied.

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Application of an Element Specific Microwave Plasma Detector to the Identification and Quantification of Micropollutants in Water

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ABSTRACT

The combination of a gas chromatograph-microwave plasma detector is studied with the objective of application to the analysis of organic micropollutants in water. With the instrument described, eleven elements (C, H, N, (O), S, P, F. Cl., Br, J, D) can be monitored simultaneously in the effluent from a gas chromatograph. Taking chlorine and bromine compounds as examples the performance parameters of the detector are described. The detection limits are 1-2 10^{-10} g/sec for C, Br, Cl, P, S and 1-6 10^{-9} g/sec for F, O, N. From the elemental ratios an identification of the individual compounds is possible. The amount of material required is in the range of 100 ng. The detector is applied to the identification and quantitative analysis of organo chlorine pollutants in surface water samples: chloroform, tri- and tetrachloroethylene, p-dichlorobenzene, hexachlorobutadiene were found in concentrations from 100-300 ng/l.

Keywords:gas chromatography, selective detectors, organohalogens, water pollution, identification, quantitative analysis.

INTRODUCTION

The quantitative analysis of organic micropollutants in water requires more and more the combination of sophisticated enrichment-, separation- and detection techniques. Whereas up to now most of the identification and quantification has been achieved by the combination of gas chromatography mass spectrometry, the application of a micro wave plasma detector (MPD) as element selective detector has found increasing interest during the last years. The use of a MPD in combination with GC was first applied by McCormack, Tong and Cooke (1965) for the analysis of organic pesticides. Since then the system has been applied to the analysis of organic Hg compounds (Bache, 1971) and to the quantitative determination of As, Se and Sb in environmental samples (Talmi, 1974; Talmi, Andren, 1974; Talmi, Norvell, 1975). The MPD proved to be a highly sensitive detector especially for volatile metal-containing species, as was recently shown by Reamer (1978) for alkyl lead compounds. Bonnekessel, Braunstein and Hochmüller (1978) were the first to apply the combination GC-

MPD for a general investigation of organic pollutants in Rhine river water. In our work the main interest was to study the applicability to volatile chlorine and bromine containing pollutants in surface waters.

EXPERIMENTAL

Instrumentation. The combination GC-MPD, as used, is shown in fig. 1. in a block diagram. A Perkin-Elmer, Mod. F22, gaschromatograph was coupled to a commercial microwave plasma detector, Mod. MPD 850 (Applied Chromatography Systems, Ltd.), which is basically the same as described by McLean, Stanton and Penketh (1973).

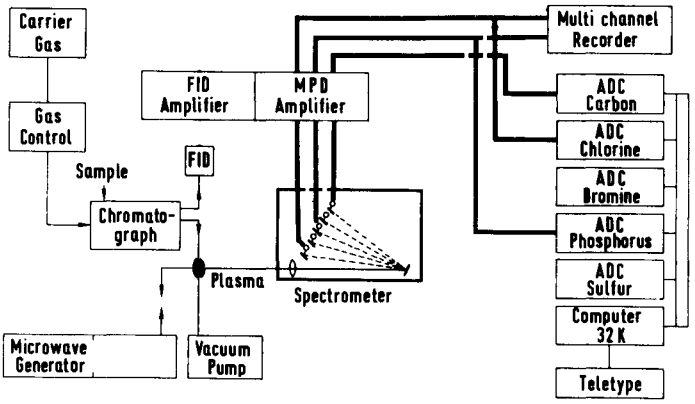

Fig. 1. Block diagram of a combination GC-MPD

The effluent from the GC is split 1:1, one part going to the FiD, the other one streaming into a transparent quartz capillary (i.d. 1 mm, 150 mm length). The plasma within the capillary was generated by a micro wave generator at 2450 MHz with a maximum power output of 200 W. The light from the plasma is passed into a spectrometer (wave length range 380-800 nm (1st order), 190-400 nm (2nd order). On eleven channels the measurement of eleven elements (C, H, N, O, S, P, F, Cl, Br, J, D) is possible via phototubes. Data registration and processing is done with a multi channel recorder and a Hewlett-Packard laboratory data system, Mod. 3353.
The GC-conditions were: column 2 m length, 4 mm i.d.; packing 3 % OV-101 on 80/100 mesh Chromosorb AWDCS; temperature: 2 min isothermal at 50°C, programmed with 6°C/min to 230°C. The concentrates from surface waters were run at 20°C (4 min isothermal) with a programming to 230°C at a rate of 6°C/min. Carrier gas was helium (99,9999 % purity) at a rate of 30 ml/min. In order to avoid deposits of carbon in the capillary oxygen was added as scavenger gas on top of the plasma head at a rate of 1 ml/min.
Sample Preparation. The stock solution containing chlorobenzene (460 mg/l), bromobenzene (563 mg/l), 1,2,4 trichlorobenzene (653 mg/l), hexachloro-

butadiene (502 mg/l), 1-chlorododecane (562 mg/l) and hexachlorobenzene (553 mg/l) in pentane was used for calibration and performance studies; lower concentrations were obtained by a tenfold dilution. The water samples under investigation were adjusted to pH-2 by addition of sulfuric acid and treated according to the closed loop stripping procedure (Grob, 1973; Stieglitz and coworkers, 1976) at 23°C for 3 hrs. The volatile organics adsorbed on a micro charcoal filter were eluted in four steps with a total of 14 µl of carbon-disulfide containing 1-chlorododecane as internal standard.

RESULTS AND DISCUSSIONS

In fig. 2. is shown a typical chromatogram of the standard solution (2 ul injected), with amounts of chlorine ranging from 194 ng (chlorododecane) to

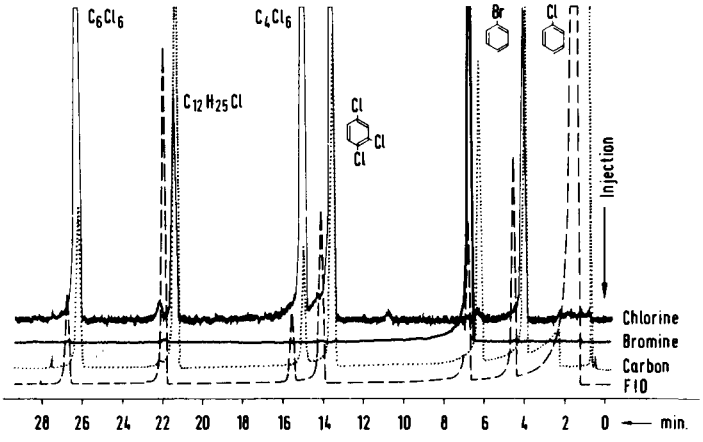

Fig. 2. Chromatogram for the elements C, Br, Cl (standard solution)

824 ng (hexachlorobenzene), the corresponding amounts of carbon being 800 ng and 280 ng respectively. The peak areas of individual elements were calculated by the lab data system and printed as reports (retention time, peak area, percentage) for each element. The reproducibility of the GC runs was tested by repeated injections; the mean standard deviation with three measurement was for the FID signals ± 2-3 % and for carbon ± 3-6 %, for chlorine ± 2-6 % and for bromine ± 6 %. The emission intensity measured as area was linearly proportional to the quantity of the element in the sample within the range of 20 ng to 800 ng of chlorine and carbon. The response in area units per nanogram was calculated and found to be independent from the species injected within ± 5-7 %.

The response, and so the sensitivity, may be increased by raising the tube current and so supplying more power to the plasma. Within the limits of the instrument the tube current can be varied from 75 mA to 125 mA without damaging the quartz capillary. This increase corresponds to an increase of sensitivity for carbon by a factor of 2, for chlorine and bromine by a factor

of 3. The detection response as a function of tube current is shown in fig. 3.

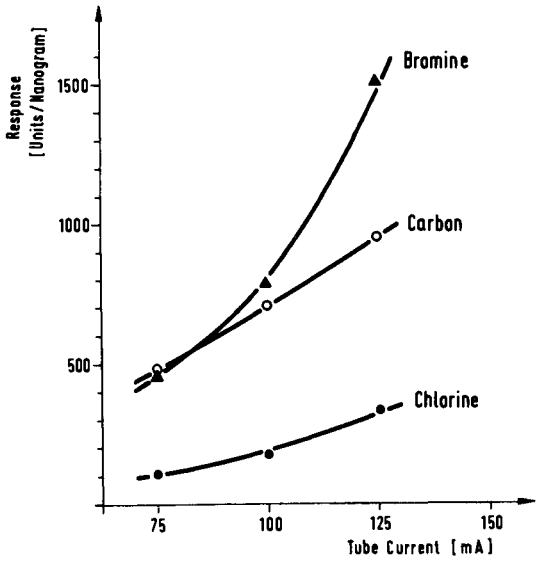

Fig. 3. Detector response as a function of tube current

Ghost Peak Correction. A non-elemental signal, referred to as "ghost" is caused by a change of the background, whenever an organic compound passes through the plasma, this change of the background is due to a carbon continum emission across the whole spectrum. The influence of this ghost signal to non-carbon-emission lines varies from element to element but all responses are proportional to the amount of carbon present in the plasma. This fact is the basis for an electronic correction device by which the ghost response can be widely eliminated with the MPD 850. The residual ghost signal was determined for all non-carbon elements by injecting a mixture of 2 µg of nonane, decane, undecane, naphthalin, dodecane and tetradecane. The "rest ghost" signal influencing the chlorine and bromine was measured and found to be less than 2 %. For analysis requiring greater accuracy this contribution may be taken in calculation and be subtracted from the non-carbon signals.

Elemental Ratios. Since the signal of the detector is linearly proportional to the amounts of the individual elements, elemental ratios can be determined from the GC-peak areas of the element. This may be done graphically by referring to calibration graphs or by calculation.

A series of chlorohydrocarbons of varying elemental ratios was injected and the ratio of peak integrals of chlorine/carbon determined. In fig. 4 these results for different tube currents are plotted versus the theoretical elemental ratio. Referring to these graphs the element ratio Cl/C for any GC-peak can be evaluated. With the use of an internal standard the determination of element ratios of unknown compounds may also be calculated using the formula

$$C/Cl = \left(\frac{A_{Co}}{A_{Cl}}\right)_{unknown} \times \left(\frac{A_{Cl}}{A_C}\right)_{STD} \times \left(\frac{n_C}{n_{Cl}}\right)_{STD}$$

where A_C and A_{Cl} are the peak area of carbon and chlorine for the unknown and

the standard compound, and n_C and n_{Cl} the number of carbon and chlorine atoms

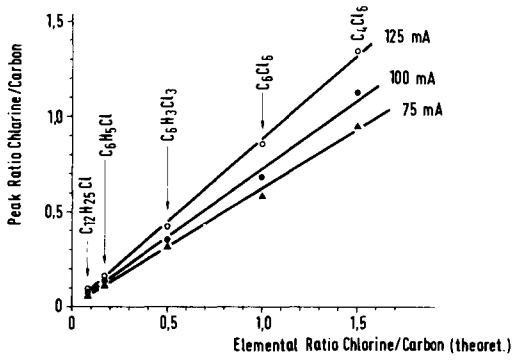

Fig. 4. Determination of elemental ratios by microwave plasma detector

in the standard. Using 1,2,4 trichlorobenzene as an internal standard (C/Cl = 2) the carbon/chlorine ratio was determined for compounds with a C/Cl ratio varying from 0,66 to 12. The results are shown in table 1. 200 to 800 ng per

Compound	Carbon/Chlorine Ratio *			
	theoretical	determined at		
		75 mA	100 mA	125 mA
Chlorobenzene	6,00	6,19	6,20	6,02
Hexachlorobutadiene	0,66	0,66	0,62	0,63
1-Chlorododecane	12,00	9,20	9,89	9,96
Hexachlorobenzene	1,00	1,07	1,02	1,00

* VS. Trichlorobenzene as Standard; amount injected 200-800 nanograms

TABLE 1. Determination of carbon-chlorine ratio by microwave plasma detector

compound were injected for this experiment. As can be seen, experimental and theoretical ratios agree very well for ratios ranging from 6.00 (chlorobenzene) to 0,66 (hexachlorobutadiene). Only for extremely high ratios 12 (chlorododecane) the deviation becomes as high as 25 %. Even at lower concentrations (20-80 ng injected) the agreement with theoretical values is satisfactory. Average data from three measurements are $6,1 \pm 0,5$ for chlorobenzene, $0,68 \pm 0,05$ for hexachlorobutadiene, $14,4 \pm 1,3$ for 1-chlorododecane and $1,0 \pm 0,1$ for hexachlorobenzene.

Limits of detection - Selectivity. The mass rate of element required to give a peak height of twice the noise level is defined as the limits of detection (L.O.D.). From the response and the noise level (0,1 mV) of the individual channels the detection limits are calculated and listed in table 2. Except for fluorine, nitrogen and oxygen the detection limits are in the range of 10^{-10} g/sec. Compared with other selective detectors such as the flame ionisation detector for carbon, the thermoionic detector for nitrogen and the flame photometric detector for sulfur and phosphorus the detection limits of the MPD are higher by a factor of several hundreds. The data given are for routine

operation with packed columns for peaks with half height widths of 10-12 sec, and can potentially be improved by using capillary columns with narrow peaks.

Element	Emission wave length Å	Detection Limits [g/sec]	Selectivity
Carbon	2478,6 (II)	$2 \cdot 10^{-10}$	1
Bromine	4704,9	$1 \cdot 10^{-10}$	590
Chlorine	4794,5	$1 \cdot 10^{-10}$	256
Phosphorus	2535,7 (II)	$1 \cdot 10^{-10}$	3800
Sulfur	5453,9	$1 \cdot 10^{-10}$	220
Fluorine	6856,0	$1 \cdot 10^{-9}$	470
Nitrogen	7468,8	$4 \cdot 10^{-9}$	not determined
Oxygen	7771,9	$6 \cdot 10^{-9}$	not determined

TABLE 2. Limits of detection and selectivity of a GC-MPD

Further improvement may be achieved by special purification of carrier and scavenger gas and elimination of all leaks.
The selectivity with respect to carbon is also shown in table 2. The data are calculated on the basis of number of mass units of carbon needed to give the same response as one mass unit of the element. In order to achieve high selectivity it is necessary to make the above mentioned ghost correction as carefully as possible. The selectivity for chlorine of 256 means e.g. that 1 ng of chlorine has the same response as 256 ng of carbon. Since the MPD is used in connection with a gaschromatographic separation of the compounds the selectivity for the mentioned elements is sufficient and even allows the discovery of traces of non-carbon elements under intense peaks.
Application to pollutants analysis. The described set up was applied to the

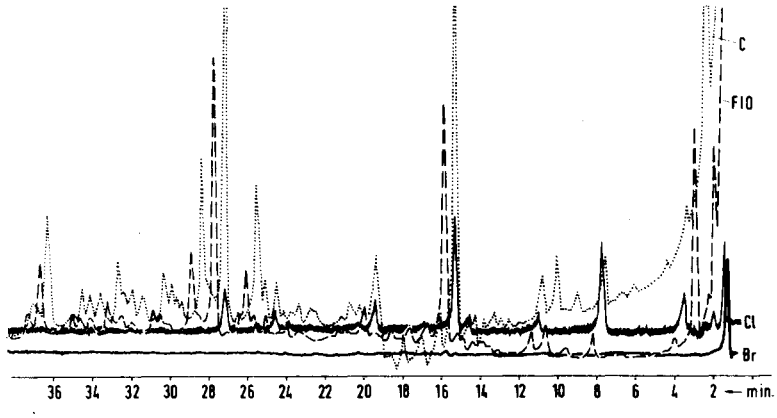

Fig. 5. Chromatogram of volatile organics from river water

analysis of volatile organic chlorine and bromine compounds which were isolated and concentrated from surface waters by the closed loop stripping technique as described by Grob (1976). In fig. 5 an element chromatogram is shown for C, Cl, Br and the FID-signal. Simultaneously monitored were also the channels for F, S, and P. From the known amounts of internal standard (1-chlorododecane) the concentrations of organic chlorine corresponding to the

individual peaks were calculated. The data are shown in table 3 as averages from three analysis. From the ratio of carbon:chlorine a tentative identification was made. The agreement of the measured ratios with the theoretical is relatively good and with the retention time as a second criterium leeds to reliable identification even at low concentrations.

Retention time [min]	Concentration organic Cl ng/l	Ratio C/Cl	Tentative Identification (C/Cl theoretical)
2,14	110±20	n.d.	Chloroform (ret.time)
4,20	120±10	0,56	Trichloroethylene (0,66)
8,07	270±30	0,53	Tetrachloroethylene (0,55)
15,40	300±50	3,6	p-Dichlorobenzene (3)
19,64	125±20	1,9	Hexachlorobutadiene (1,5)
27,41	–	11,8	1-Chlorododecane (std)

Sample: CS_2 - Eluate, 2 l Riverwater (Rhine)

TABLE 3. Identification and quantitative determination of water pollutants

The main chlorine compounds were tetrachloroethylen and p-dichlorobenzene in concentration of 270-300 ng/l, and chloroform, trichloroethylen and hexachlorobutadiene with 110-125 ng/l. No bromine, fluorine, sulfur and phosphorus compounds could be found above detection limits.
The absolute amount injected was from chloroform, trichloroethylen and hexachlorobutadiene around 20 ng, and from tetrachloroethylene and dichlorobenzene ca. 60 ng.

CONCLUSIONS

From the data so far available it can be concluded, that the application of the microwave plasma detector shows interesting potential for the analysis of organic pollutants. In combination with efficient separation and enrichment techniques a fast qualitative information about the presence of heteroelements like halogens, sulfur, phosphorus and nitrogen can be obtained. As a selective detector to various elements its response is proportional only to the amount of element present and is independent on bond-character or oxidation state. It is therefore the best approach to a problem-oriented, quantitative analysis. In combination with low resolution mass spectrometry the knowledge about the presence of heteroatoms such as sulfur, oxygen and especially nitrogen is very helpful and advantageous facilitating the interpretation of mass spectra. From the ratio of the respective elemental peak area the elemental composition can be evaluated, leading to an identification of the compounds.

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Multicomponent UV Spectral Analysis of Aquatic Organics

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ABSTRACT

The utilization of multiwavelength ultraviolet absorbtion measurements for analyzing multicomponent mixtures of aquatic organic material is described. Applications are visualized in monitoring organic pollutant levels in natural bodies of water, process control in wastewater treatment and water treatment. The methodology for analyzing measurements of UV absorbance spectra and elemental composition of multicomponent mixtures to determine the concentration in terms of their major constituents is described.

Key Words: Aquatic Organics, UV Absorbtion, Characterization, Organic Pollutants.

INTRODUCTION

Myriad organic chemical constituents are continuously being added to the aquatic environment from industrial point sources and non-point sources such as agriculture, silviculture, and runoff from the land. While the bulk of this influx represents natural organic decay products, there is concern about the rapidly increasing influx of man-made compounds that resist biological decomposition and therefore tend to accumulate in the aquatic environment. The potential hazards of man-made compounds to human health and the ecosystem depend on their structure and composition; the effects of many have not been defined. Nevertheless, government agencies in the United States have taken steps to monitor and regulate their inflow and limit the concentrations of organic matter in drinking water.

Detection and monitoring of organic compounds turns out to be a herculean task. Measurement of trace contaminants requires sophisticated and expensive instrumentation (gas chromatography, mass spectrometry and a variety of concentrating and fractionation techniques), as well as highly trained specialists. It is clearly not economically feasible to continuously monitor thousands of

locations for numerous organic compounds. The problem of monitoring is further complicated by the fact that organic constituents which are normally present from natural sources may also be detrimental. Natural aquatic organics have been linked to the transport-solubilization of chemicals of anthropogenic origin; they have also been implicated as precursors of carcinogens (halomethane formation during chlorination).

There is therefore an urgent need for new analytical methods that can be used routinely, rapidly, simply, continuously, and at low costs to measure specific pollutants or classes of pollutants. The available non-specific tests for biochemical or chemical oxygen demand or total organic carbon are inadequate because they do not give sufficient information about the composition of pollutants.

One promising technique that has received relatively little research attention is the application of multiwavelength ultraviolet (UV) absorbance measurements. Absorbance measurements at a series of wavelengths can be used to determine chemical structure and concentrations of specific compounds or classes of compounds. In principle, the analysis involves superposition of absorbance spectra of pure compounds or classes of compounds to match the absorbance spectrum of the mixture and to estimate their respective concentrations. This paper describes the initial results of research aimed at a) utilizing multiwavelength UV absorbance as a nonspecific measure of organic pollutant concentrations in natural waters and/or wastewaters, b) developing the methodology for analyzing multiwavelength absorbance measurements of aquatic organics to obtain specific compound information.

State of the Art

Applications of UV measurements in the water pollution control field have been reviewed by Dobbs (1972). Absorbance measurements at 254 nm have been correlated with organic carbon concentration (Dobbs, 1972, Symons, 1975), potential oxygen demand (Mrkva, 1971) and used for monitoring industrial effluents (Bramer, 1966). Concentration of organic carbon (TOC) and A_{254} are strongly correlated for specific rivers (Dobbs, 1972; Briggs, 1968) but finished drinking waters from 80 different cities and hence different raw water sources exhibited considerable scatter (Symons, 1975).

Applications of UV spectrophotometry for analyzing natural products are well documented (Scott, 1964, Morton, 1975); it is probably the most widely used analytical measurement; however, quantitative measurements are usually made at a single wavelength at or near maximum absorbance. Analysis of multiwavelength measurements has not been widely used. Arends *et al.* (1964) analyzed mixtures of arylsulfonic acids with reference to the pure compound spectra of each constituent. Metzler *et al.* (1977) described a mathematical analysis for resolving spectra of multicomponent mixtures and showed how this approach could be applied to evaluating pK values. Superposition of absorbance spectra and measurement of difference spectra relative to known model compounds has been used to characterize lignin and its degradation products (Morton, 1975). Kankare (1970) calculated molar absorbtivities and equilibrium constants from multicomponent spectrophotometric data. However, the computerized data analysis system proved unwieldy due to excessive data storage requirements.

Review of the published information indicates that multiwavelength analysis of mixtures is a potentially useful technique for obtaining quantitive informa-

tion about their constituents. However, the methodology for data gathering and interpretation of results for multicomponent mixtures needs to be developed.

Mathematical Technique for Analysis of Multiwavelength Absorbance and Elemental Composition Data

Analysis of the absorbance spectrum of mixtures to elucidate their composition is based on the assumptions that absorbance follows Beer's Law, that there is no interference between chromophores, and absorbance is additive; the latter implies that the absorbance of mixtures is equal to the sum of absorbance contributions of each chromophore. The procedure is conceptually simple; absorbance spectra of pure compounds are measured independently and used to simulate the measured absorbtion spectrum of the mixture by summation of the constituent spectra at the appropriate concentration as illustrated below:

$$\sum_j A_{ij} \cdot X_j = b_i$$

Absorbance per unit concentration of each pure compound or constituent is represented by $A_{i,j}$ where subscript i identifies the wavelengths and j identifies the compound; X_j represents the concentration of the j^{th} constituent; b_i is the absorbance of the mixture at wavelength i. The summation is made at any arbitrary number of wavelengths i. The $A_{ij}X_j$ values describe an i by j matrix and the absorbance values b_i define an i component column vector. The elemental composition data is analyzed in a similar manner because mass of carbon, hydrogen, oxygen and nitrogen are additive. Material balances on elemental organic carbon, hydrogen, oxygen and nitrogen are calculated as shown below, thus adding four rows to the i by j matrix and four corresponding terms to the column vector b. TOC, TOH, TOO, TON represent the concentration of organic carbon, hydrogen, oxygen and nitrogen per unit of concentration for each of the j constituents and the mixture b. The objective is to calculate values of the row vector X_j so as to minimize the summation:

$$\sum_j TOC_j \cdot X_j = b_{TOC} \qquad \sum_j TOH_j \cdot X_j = b_{TOH}$$

$$\sum_j TOO_j \cdot X_j = b_{TOO} \qquad \sum_j TON_j \cdot X_j = b_{TON}$$

$$\sum_i \{ b_i - \sum_j A_{ij} X_j \}^2 + \{ b_{TOC} - \sum_j TOC_j X_j \}^2 + \{ b_{TOH} - \sum_j TOH_j . X_j \}^2 ----$$

subject to constraint $X_j \geq 0$. The constraint that $X_j \geq 0$ precludes negative values that would imply emission of light energy or removal of elements.

Solution techniques were briefly studied to find an algorithm that converges on a reasonable solution in a finite number of iterations. The Non-negative Least Squares subroutine (NNLS) described by Lawson (1974) was found to be the most effective. The NNLS subroutine computes a solution vector X for any m by n matrix and its m component column vector b. The program always calculates a solution but the solution is non-unique if the rank of the matrix is $< n$. The algorithm assumes that the integers of the m by n matrix and the m component column vector are given. N component vectors w and z provide working space. Index sets P and Z are defined and modified in the course of execution of the algorithm. Variables indexed in set Z are held at value zero, variables in the set P are different from zero but non-positive variables are made positive or moved to Z. On termination, the subroutine prints out the concentrations X_j, absorbance contributions $A_{ij}X_j$ of

each constituent, the total absorbance, and the euclidian norm of the solution vector.

Hypothetical Model Compound Studies

The effectiveness of the algorithm for calculating the concentration of constituents that best simulate the absorbance of a mixture was test simple hypothetical absorbance-wave number correlations illustrated in Fig. 1.

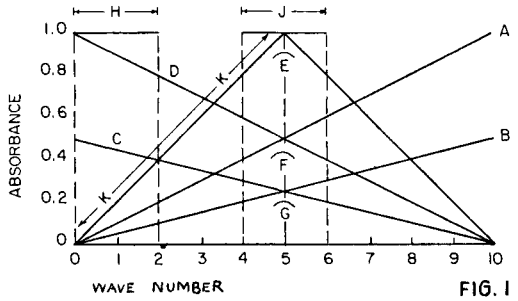

FIG. 1

Simple absorbance-wave number relationships were used to facilitate interpretation. A and B absorbances increase with wave number and are linearly related to A for the first five wave numbers but then becomes zero. C and D are linearly related but are inverses of A and B, whereas E, F and G are linearly related combinations of A and C. H and J are step functions designed to evaluate the program's effectiveness for handling constituents with narrow absorbtion bands. The absorbance-wave number data of A through J were provided as input to the subroutine and represent the inventory of possible constituents. Absorbance spectra of arbitrary mixtures of A through K were evaluated. Analysis of the simulation tests leads to the following conclusions: 1) the algorithm calculates an exact match of the whole absorbance spectrum for multicomponent mixtures. Values of the Euclidian Norm (EN) are 10^{-13} or less; 2) concentrations of constituents are calculated exactly for mixtures consisting of constituents whose absorbance spectra are not linearly related; 3) mixtures containing constituents whose absorbance-wave number spectra are linearly related over the whole range are lumped together and only the constituent with the highest absorbance is identified. For example, mixtures of A and B are reported as an equivalent concentration of A because the latter has higher absorbance. Similarly E, F and G are lumped together and reported as an equivalent concentration of E; 4) the shape of the absorbance-wave number correlation of the mixture has no effect on the calculations; mixtures with several maxima, minima, or completely featureless and uniform spectra are matched exactly; 5) if the input values of the absorbance of the mixture are in error, the algorithm calculates a best fit. This may result in identification of spurious constituents. The subroutine is sensitive to small errors. EN values smaller than 10^{-2} are indicative of small mismatches that may result from errors in the third significant fig. of one of the absorbance values; the calculated error in concentration is less than 0.3%. EN values in the 10^{-1}-10^{-2} range result from an error in the second significant fig. of one of the absorbance values (1-10%) and give errors up to 6% in calculated concentrations. Incorrect values of absorbance values of the order of 50% may

result in substantial mismatching and concommittantly large errors in calculated concentrations. Spurious compounds are brought into the calculation. The calculated EN values may therefore be used as one measure of the reliability of the fit; 6) if input errors lead to mismatching in a specific wave band, spurious constituents with the highest absorbance are brought into the solution; 7) if mismatching is evident, a series of tests with progressive elimination of inventory constituents may be useful for characterizing the absorbance "errors." The absorbance "error" may reflect the fact that a critical constituent is not available in the data bank inventory or that the absorbance input data are erroneous.

Algorithm Evaluations Using Naturally Occurring Model Compound

Mixtures of six pure compounds associated with decay of biomass were studied in a similar manner. Absorbance spectra of 10 mg/l solutions of each compound were measured with a Beckman Model #26 Spectrophotometer using one centimeter cells and provided as input to the subroutine along with their respective elemental composition; compound spectra, as illustrated in Fig. 2 have one or more maxima; however, combinations of the model compounds give relatively featureless absorbance spectra that resemble natural water spectra. Absorbance values were specified in two nanometer increments for the 230-348 nm range; elemental composition was specified in units of mg/l. A series of arbitrary mixtures were tested. The subroutine calculated an exact match of all absorbance values, correctly calculated the concentrations of each constituent and gave low EN values. Fig. 3 shows typical results for the Case 3A mixture which has pronounced maximum at intermediate wavelengths. The low EN value 10^{-13} is indicative of exact matching. Performance of the algorithm for simulating mixtures when one of the constituents is not available in the data inventory of compounds was tested by deleting tannic acid. The results are shown in Figures 4 and 5. When tannic acid is a major constituent of the mixture, calculated absorbance values and elemental composition are poorly matched (Fig. 4). EN of 1.6 is a clear indication of poor fit. Fig. 5 shows that a lower EN value and closer matching of absorbances and concentrations is obtained when tannic acid is present at lower concentrations. Similar calculations were made with salicylic acid eliminated from inventory. On the basis of these tests it was concluded that the algorithm is an effective tool for analyzing multiwavelength spectra provided that the necessary constituents are available in the inventory data bank. Relatively featureless spectra are correctly matched. If a major constituent compound is not available in inventory, the best fit calculations usually identifies all other major constituents though not at their correct concentration. Spurious compounds are identified when large mismatching occurs. Values of EN >0.5 are indicative of major mismatching. Comparison of the calculated and measured spectrum gives insight on the absorbance spectrum of the constituents that are missing from inventory. Conversely, wavelength regions of excess absorbance are indicators of the absorbance spectrum of spurious compounds or excess contributions of actual constituents. Sequential addition and deletion of inventory compounds is therefore useful in pinpointing spurious compounds.

Inventory of Constituents for Analysis of Aquatic Organics

Surface waters and sewage contain a large diversity of organic constituents of natural origin (decay of biomass) and man related sources. This poses a problem because there is a practical limit to the number of pure compounds

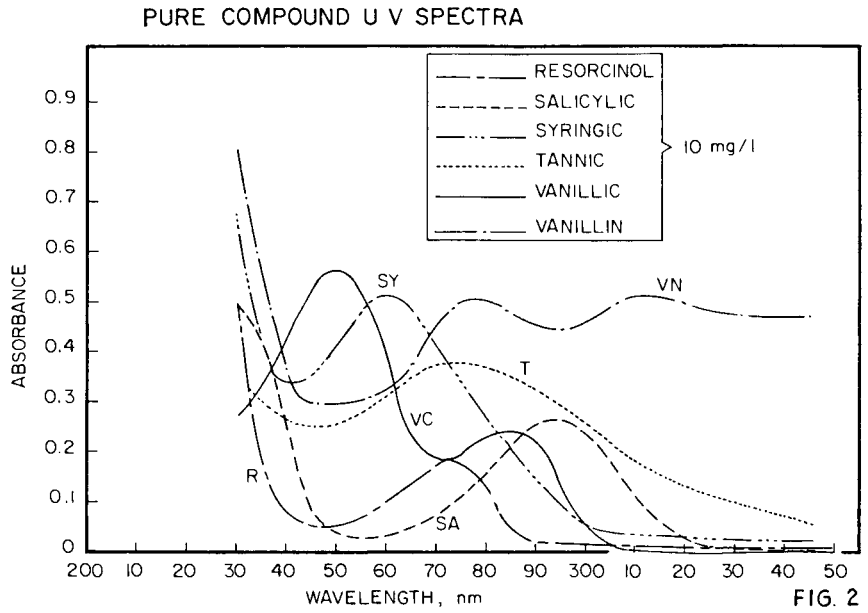
PURE COMPOUND U V SPECTRA
RESORCINOL
SALICYLIC
SYRINGIC
TANNIC
VANILLIC
VANILLIN
10 mg/l
ABSORBANCE
WAVELENGTH, nm
SY
VN
T
VC
R
SA

FIG. 2

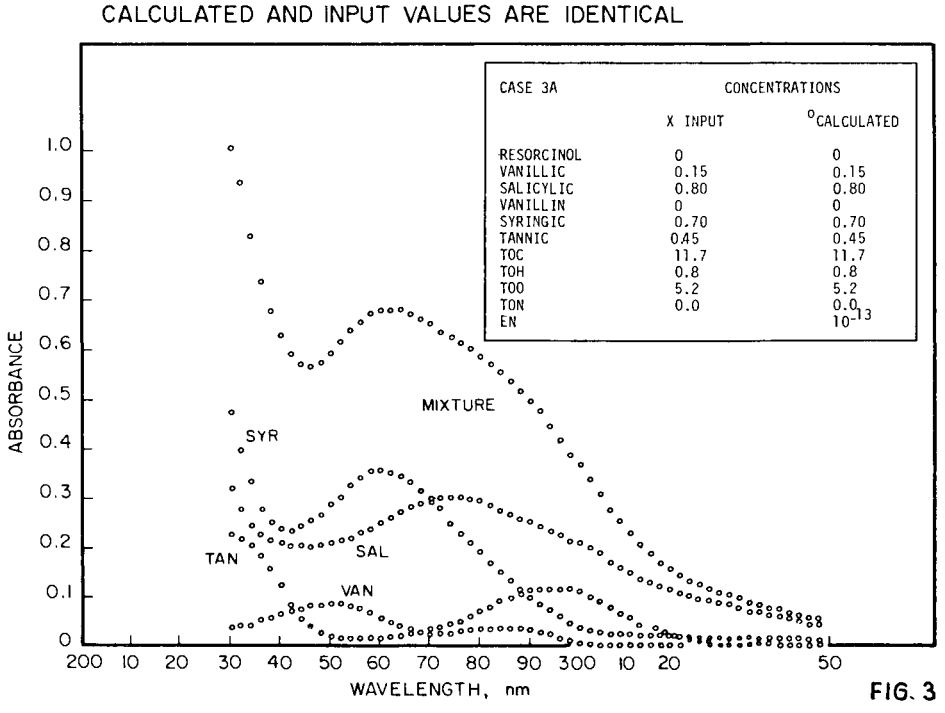
CALCULATED AND INPUT VALUES ARE IDENTICAL
CASE 3A
CONCENTRATIONS
X INPUT
°CALCULATED
RESORCINOL 0 0
VANILLIC 0.15 0.15
SALICYLIC 0.80 0.80
VANILLIN 0 0
SYRINGIC 0.70 0.70
TANNIC 0.45 0.45
TOC 11.7 11.7
TOH 0.8 0.8
TOO 5.2 5.2
TON 0.0 0.0
EN 10-13
ABSORBANCE
WAVELENGTH, nm
MIXTURE
SYR
TAN
SAL
VAN

FIG. 3

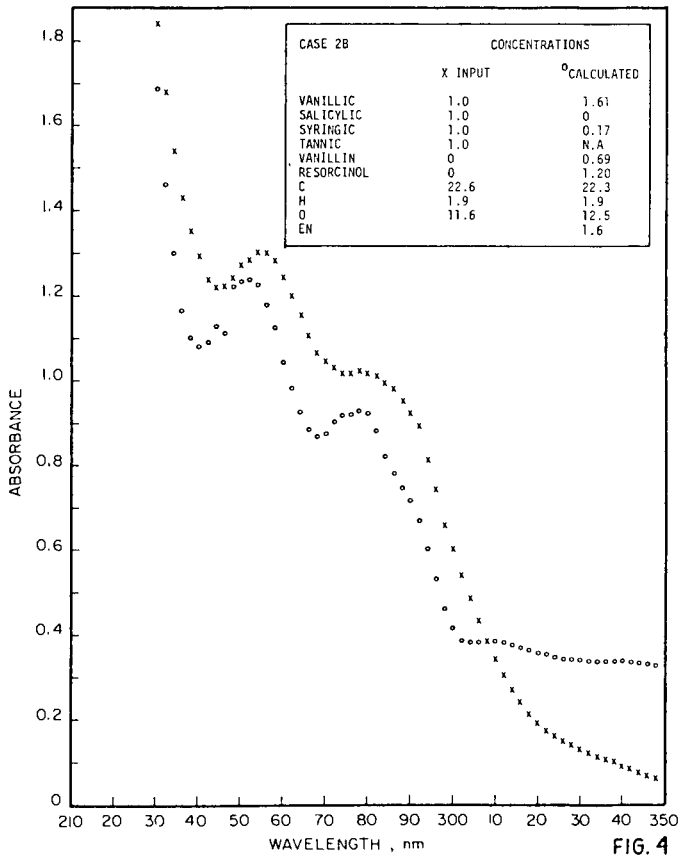

FIG. 4

TANNIC MISSING FROM INVENTORY

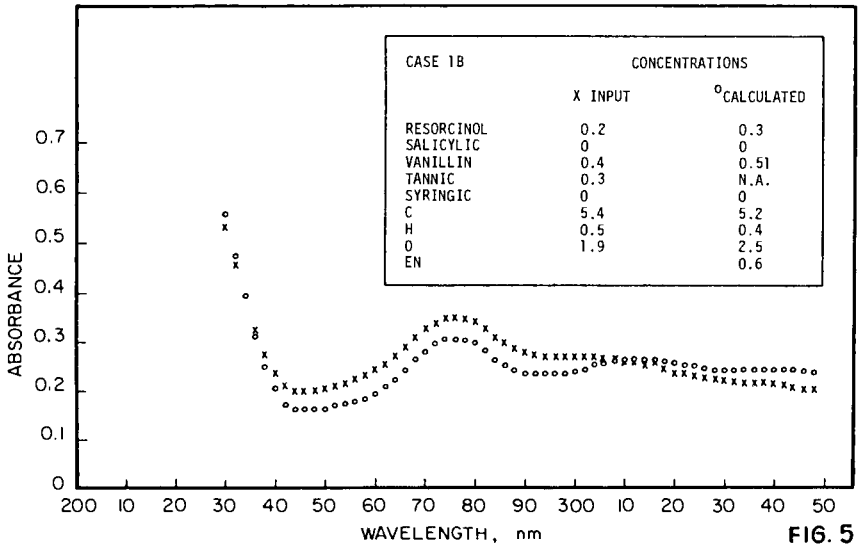

FIG. 5

that can be considered. Ideally, the subroutine's inventory should include all the major constituents that are likely to be present. As regards the natural aquatic constituents, information about their chemical composition, structure, and spectrophotometric characteristics is incomplete. Decay Products of biomass include common metabolites such as amino acids-proteins, carbohydrates, and lipids but most of these compounds are rapidly metabolized by aquatic microbes and are therefore transients; concentrations are likely to be very low except in recently polluted waters. By contrast the humic-fulvic materials that are leached from soil organics consist of biochemically refractory materials that include lignin and tannin related materials. Typical degradation products of lignin include mono, di, and tri methoxy, carboxy, and propyl substituted phenols. Tannic acid derivatives are likely to be present in waters that drain areas undergoing vegetative decay. Humic and fulvic acids represent the long-term products of decay and condensation of intermediates. They are believed to be polymers of phenolic constituents bonded by covalent carbon-carbon and carbon-oxygen bonds and multiple hydrogen bonds (Schnitzer, 1972; Gieseking, 1975). The residual organics in biologically treated sewage are remarkably similar to humic-fulvic leachates from soils (Rebhun, 1971). However, raw sewage has large concentrations of common metabolites (Hunter, 1965) that are likely to undergo decomposition.

As regards the absorbance contributions attributable to man-made compounds that accumulate as aquatic organics, some a priori knowledge of the types of compounds likely to be present is required. Information about the inflow of industrial wastes is usually available from regulatory agencies and gives insight on the major constituents that should be included in the data bank inventory. The presence of very low concentrations (micrograms per liter) of any specific compound is not detectable by direct absorbance measurements of the whole water sample. For example, the absorbance of 1.0 µg/l of PCB which has a high extinction coefficient can not be detected in a background of 10 mg/l of naturally occurring organic matter because the latter dominates the absorbance due to its 10,000 fold higher concentration. It is therefore anticipated that some form of sample pretreatment to fractionate and/or concentrate classes of compounds will be utilized. Adsorbtion on high surface area resins, solvent extraction and physical separation methods may be used. Such techniques are presently being studied in many laboratories including our own. One of these methods, use of ultrafiltration membranes to size fractionate and concentrate organics from Mississippi River water has been evaluated and preliminary results reported at the meeting. The results are not included here because of space limitations.

The initial inventory of pure compounds shown in Fig. 6 was selected for analyzing Mississippi River water and sewage. It includes degradation products of lignin-humic-fulvic acids as well as compounds representative of recent biomass additions, namely amino acids and other nitrogen containing compounds whose absorbance contribution might be significant. The choice of specific compounds focused on finding compounds representative of a class of similar compounds and commercial availability. Absorbance measurements and elemental composition concentrations of 10 mg/l solutions were inventoried and used for matching calculations.

Mississippi River Water Analysis

Screening studies were carried out on water samples from five locations on the Upper Mississippi River (MRW) representing unpolluted headwaters, agri-

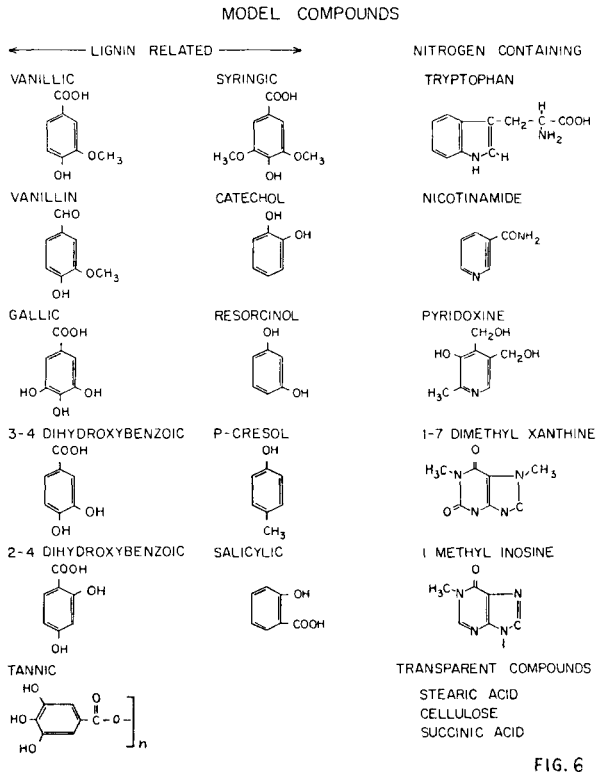

FIG. 6

culturally developed regions, and bracketing the industrialized metropolitan area of Minneapolis and St. Paul. Sewage (SEW) representing progressive stages of treatment at the metropolitan treatment plant were also tested. TOC and UV absorbance spectra were measured over the 200-350 nm range at ambient pH. Results of the matching calculations are summarized below; detailed results will be published in a separate report. Simulation studies using 14 of the constituents (no transparent compounds) listed in Fig. 6 are summarized in Table 1a. Sample locations are identified by their Upper Mississippi River Mile Index (UM); 1365 is the source region near Bemidji, 1292 is near Grand Rapids, 914 is near Royalton and includes the heavily farmed region, 895 and 698 bracket the industrialized region of Minneapolis and St. Paul; 698 represents the most pollution stressed part of the river. Raw wastewater was obtained at the Minneapolis-St. Paul sewage treatment plant as were samples after primary treatment, secondary treatment and final effluent. Primary treatment removes only suspended solids. Secondary treatment includes activated sludge treatment and clarification; the effluent is chlorinated before discharge to the Mississippi River. MRW samples gave EN values of 0.04 -0.1 which indicate marginal fitting. Lignin was identified as a major constituent in all samples. Gallic acid is also identified as a major constituent increasing from 0.95 to 2.51 mg/l in the downstream direction. Vanillin and 2-4 dihydroxybenzoic acid show a similar trend; 1-methyl inosine and 1-7 dimethyl xanthine (1-7 DMX) were identified at low concentrations; salicylic acid was identified at low concentrations in the downstream samples.

Raw sewage and primary effluent were poorly matched, EN values of 0.2-0.3,

Table 1a

Simulation of Mississippi River and Sewage Samples with 14 Constituent Inventory

(Jan., 1978)

	E.N.	Lignin	Tannic	Vanillic	2-4DHBA	3-4DHBA	1-Methyl inosine	1-7 DMX	Syringic	Resorcinol	Gallic	Vanillin	Cresol	Catechol	Salicylic
Mississippi River, 1/78					Concentration of Constituents, mg/l x 10^{-1} (mg/100 ml)										
UM 698	0.10	0.186	0	0	0.161	0	0.055	0.074	0	0	0.251	0.147	0	0	0.043
UM 895	0.09	0.241	0	0	0.156	0	0.022	0.081	0	0	0.220	0.105	0	0	0.010
UM 914	0.09	0.233	0	0	0.149	0	0.026	0.070	0	0	0.233	0.100	0	0	0.012
UM 1292	0.05	0.170	0	0	0.072	0	0	0.032	0	0	0.132	0.036	0	0	0
UM 1365	0.04	0.126	0	0	0.054	0	0.074	0.028	0	0	0.095	0.027	0	0	0
Wastewater															
Raw	0.26	0.665	0	0	0.473	0	0	0.246	0	0	0.810	0.629	0	0	0.100
Primary	0.27	0.594	0	0	0.483	0	0	0.269	0	0	0.701	0.625	0	0	0.075
Secondary	0.03	0.368	0	0	0.010	0	0.159	0	0	0	0.165	0.065	0	0	0.034
Effluent	0.04	0.311	0	0	0.032	0	0.114	0	0	0	0.190	0.054	0	0	0.012

Table 1b

Simulation of Mississippi River and Sewage Samples with 13 Constituent Inventory

Mississippi River, 1/78					Concentration of Constituents, mg/l x 10^{-1} (mg/100 ml)										
UM 698	0.19	0	0.323	0	0.079	0	0.288	0	0	0	0.144	N.A.	0	0	0
UM 895	0.15	0	0.224	0	0.083	0	0.230	0	0	0	0.186	N.A.	0	0	0
UM 914	0.15	0	0.213	0	0.081	0	0.226	0	0	0	0.189	N.A.	0	0	0
UM 1292	0.06	0.072	0.072	0	0.044	0	0.078	0	0	0	0.130	N.A.	0	0	0
UM 1365	0.05	0.049	0.057	0	0.032	0	0.069	0	0	0	0.097	N.A.	0	0	0
Wastewater															
Raw	0.78	0	1.339	0	0.138	0	0.863	0	0	0	0.343	N.A.	0	0	0
Primary	0.78	0	1.324	0	0.144	0	0.816	0	0	0	0.302	N.A.	0	0	0
Secondary	0.09	0.175	0.138	0	0	0	0.310	0	0	0	0.033	N.A.	0	0	0
Effluent	0.08	0.131	0.106	0	0.007	0	0.276	0	0	0	0.093	N.A.	0	0	0

lignin, vanillin, 2-4 DHBA, 1.7 DMX and gallic were identified as major contributors. By contrast, treated sewage EN values and constituent distributions are similar to MRW samples. Raw and primary samples identified 1-7 DMX and 2-4 DHBA, whereas treated wastes showed 1-methyl inosine and negligible concentrations of 2-4 DHBA. Table 1b lists the calculated distribution when vanillin was deleted from inventory; its elimination was prompted by observation that its UV absorbance is strongly pH dependent in the pH range of natural waters. Comparison of the results shows a dramatic shift from lignin to tannic acid. 2-4 DHBA and gallic concentrations are reduced but 1 methyl inosine increased; 1-7 DMX was not identified. The EN values are slightly larger in all cases.

Analysis of the simulation calculations give some interesting clues to identification of the major constituents of aquatic organics. Identification of a specific constituent is viewed as indicating the presence of a class of compounds with similar absorbance characteristics. By analogy, spectra of the constituents that are not identified are probably indicative of the absence of that class of compounds. The high concentrations of gallic acid indicate that its absorbtion spectrum is important; it occurs in all the samples. The identification of lignin is not unexpected because of the inflow of paper mill wastes in the upper reaches of the Mississippi River. The maximum lignin concentrations upstream of the metropolitan area is consistent with this observation. However, the ubiquitous presence of color in MRW led to the expectation that tannic acids would be identified as major constituents. The absence of tannic acid was therefore surprising and a variety of tannic extracts from different sources will be evaluated to see whether their spectra are significantly different. Lignins from different sources will also be evaluated. The interacting effects of vanillin, tannic and lignin suggest that there is considerable overlap between these constituents. The absence of phenol, catechol, resorcinol, syringic and vanillic acid spectra indicates that the absorbance spectra of these classes of compounds are not major contributors. However, a more complete assessment and interpretation of the simulation studies requires additional information about the physical chemical properties of the aquatic organics. Such information is being obtained in an ongoing research program aimed at characterization and identification of major constituents of MRW samples in conjunction with fractionation and separation schemes to facilitate measurements (Maier, 1978).

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CONCLUSIONS

There is an urgent need for new analytical methods that can be used routinely, rapidly, simply, continuously and at low cost to measure specific pollutants or classes of pollutants. A study aimed at utilizing multiwavelength UV absorbtion measurements to analyze aquatic organics was therefore carried out. The focus for the study evolved from published information showing that absorbance and organic concentrations are strongly correlated.

An algorithm for analyzing multiwavelength-multicomponent mixtures was developed and computer programmed. It allows calculating the concentration of each constituent in a mixture from the measured values of UV absorbance and elemental composition of the mixture. The algorithm matches the absorbance spectrum of the mixture by superposition of the absorbance spectra of its constituents. The latter are provided as input in the form of an inventoried data bank. The algorithm has been validated using hypothetical compounds, with pure model compounds representative of lignin decay products and tested on Mississippi River water and sewage. The results show that the absorbance spectra and elemental composition are perfectly matched and constituent concentrations identified correctly provided that the necessary constituents are available in inventory. Calculated values of the euclidian norm for the solution vector give an indication of the fit of the calculated versus actual values. If some of the mixture's constituents are not available in inventory, the program calculates a best fit composition. This best fit composition usually includes all the major constituents actually present but not at their correct concentration; it may also identify spurious compounds that are not present in the mixture but are available in the data bank inventory. The extent of substitution and concentration errors depend on the relative importance of the absorbance and composition of the non-available compound.

Preliminary matching studies of Mississippi River water and sewage using a very small data bank of 13 "compounds" have identified four major constituents and two minor constituents. Identification of lignin, gallic and vanillin are indicative of the presence of classes of compounds whose absorbance spectra are similar to those of the identified compounds. Equally important information is obtained from the fact that a number of inventory compounds such as catechol, resorcinol, syringic and vanillic acid are not identified because this means that compounds with similar absorbance spectra are not present in the river water and sewage.

Further studies are in progress. A variety of techniques for prefractionating the organic materials by molecular size, polarity, and solubility have been utilized. Results on the analysis of molecular size fractionated materials have been briefly summarized in the verbal presentation but were deleted from this paper because of space limitations.

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Microflora and Microfauna of Waste Waters

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ABSTRACT

Heterogeneous microbial populations from activated sludge of industrial origin were maintained on a synthetic waste water medium using a conventional continuous culture apparatus. A manometric bioassay was used as a rapid test of the biodegradability and toxicity of several industrial sewages. Experiments have been performed in order to achieve a balance between the microbial populations (bacteria and protozoa) and the substrates to be degraded. It has been possible to isolate some microbial species adapted to grow on several toxic substances added as the sole carbon source. As a practical implication of this work we are trying the implantation of the microbial population adapted to some toxic substances into an activated sludge system.

INTRODUCTION

A large variety of microorganisms (mainly bacteria and protozoa) are directly involved in water pollution control, either participating in the so-called "self-purification" reactions or in the treatment plants designed in such a way that they can provide the most favorable environment for the microorganisms (Wuhrmann, 1964). Three successive major steps may be considered in a sewage treatment plant: the primary or mechanical treatment, the secondary or biological treatment and the tertiary or chemical treatment, (Porges, 1960). The microorganisms in the activated sludge process form an ecosystem in which heterogeneous populations of bacteria and protozoa are submitted to continuous culture conditions. Each microorganism present in the activated sludge plays a role (for good or for bad) in the biological process and the quality of the effluent will depend on the predominance of the appropriate microorganisms. For this reason it is important to know that the presence of some chemical agents such as metallic ions, extreme hydrogen ion concentrations and certain organic compounds may produce imbalances in the activated sludge ecosystem leading to the disappearance of some esential microbial species. Experiments were undertaken to study some of the microflora and microfauna imbalances in activated sludges from the waste water treatment plant of the petrochemical industry ENPETROL at Puertollano (Spain).

RESULTS AND DISCUSSION

Continuous culture of the activated sludge.- Samples of activated sludges were cultivated in a conventional continuous culture apparatus using a synthetic waste water medium with glucose as growth limiting substrate. At pH values below 6, filamentous forms developed (Fig. 1) and both Zoogloea sp, and ciliated protozoa disappeared. Similar results were found using glucose concentrations above 1000 ppm.

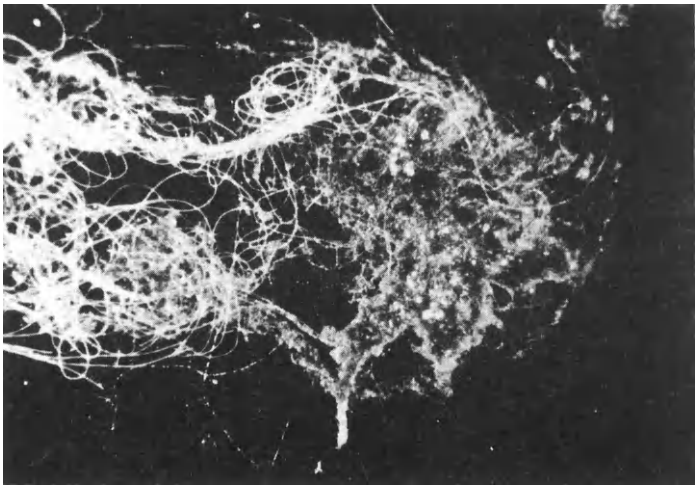

Fig. 1. Filamentous microorganisms.

Inhibition and assimilation tests.- Inhibition and assimilation tests were performed with four waste water samples from different factories that we call A, B, C and D. Samples of activated sludge were seeded on Petri dishes containing casitone-glycerol-yeast extract medium (Piked and co-workers 1972). Four wells sized 7 mm in diameter were made in each plate by using a sterile n°4 cork borer, and 0.1 ml samples of A, B, C and D were allowed to diffuse on their respective wells. After 24 h of incubation at 25°C it was found that the waste waters A and B caused a growth inhibition of the activated sludge microflora (Fig. 2). The inhibitory effect disappeared when the samples A and B were diluted to 1:4 with distilled water. For assimilation tests activated sludge were seeded on Petri dishes containing a basal medium without organic source and 0.1 ml samples of A, B, C and D were allocated into their respective wells. It was found that waste waters C and D contained enough biodegradable substances to be used as the sole carbon source by the microflora of the activated sludge (Fig. 3).

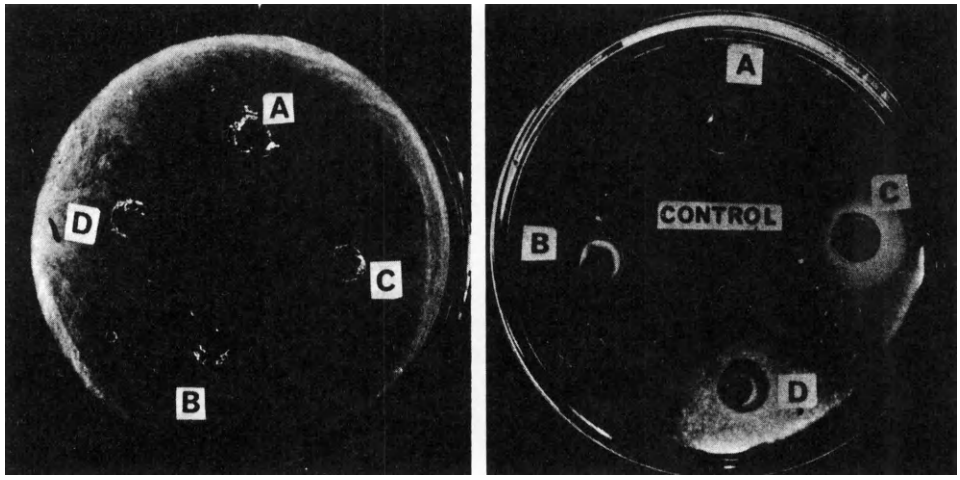

Fig. 2-3. Inhibition and Assimilation tests.

Manometric studies.- Conventional Warburg techniques (Umbreit and co-workers 1957), were used to determine the oxygen comsumption of the microorganisms in the presence of A, B, C and D as sole carbon source. It was found that the activated sludge microorganisms can oxidize the waste waters A and B when these are appropriately diluted, whereas at high concentrations no oxygen uptake can be observed even when glu cose was added simultaneously. In fig. 4 are showed the microliters of oxygen uptake by a suspension of activated sludge in the presence of waste water A. Similar results were obtained with waste water B. On the other hand, waste waters C and D were easily oxidized. In Fig. 5 are showed the results obtained with waste water C.

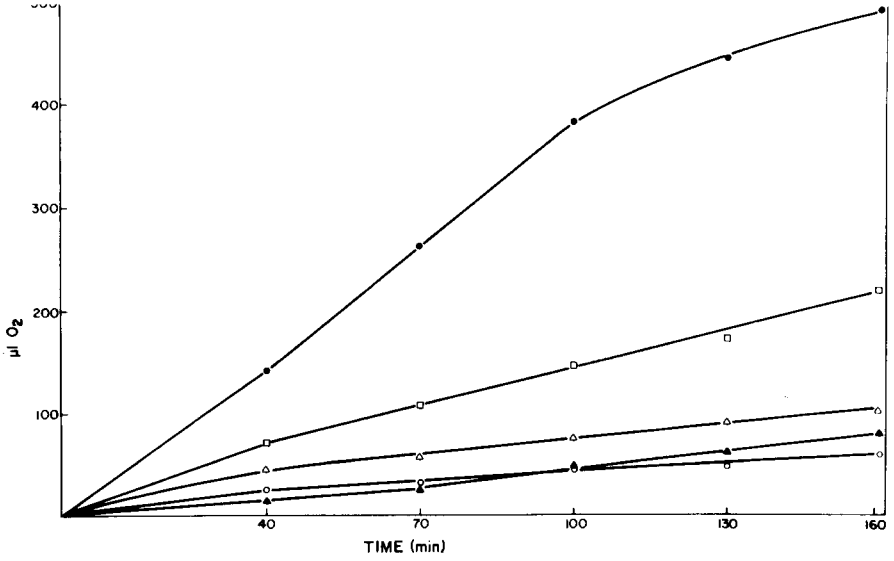

Fig. 4. Sample A.- V_F=3ml; activated sludge 4.8 mg; substrate: 0.8 mg, (□); 4 mg, (○); 4.0 mg + 1.8 gluc, (Δ); 1.8 mg gluc, (●); endogenous (▲).

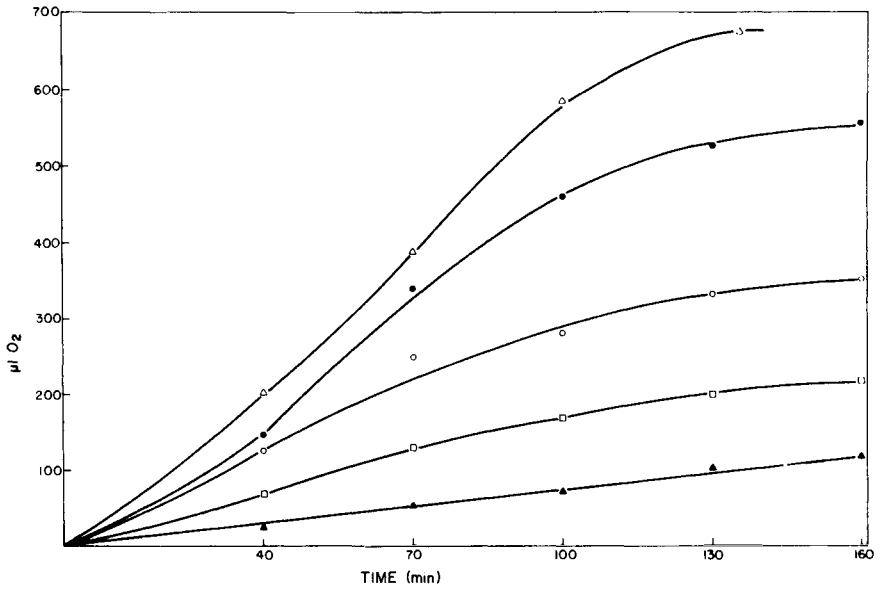

Fig. 5. Sample C.- V_F=3ml;activated sludge 5.4 mg; substrate: 0.8 mg, (□); 4 mg, (o); 4.0 mg + gluc, (Δ); 1.8 mg gluc, (●); endogeneous (▲).

From manometric studies it seems possible to establish the maximal concentration of inhibitory substances present in a waste water (for instance A and B) which may be tolerated by the organisms of the activated sludge.

Adaptation experiments.- In many cases it could be convenient to estimulate the development of microbial populations capable to degrade some substances classified as microbiologically "hard" compounds. This can be achieved by adapting the microorganisms to grow on those "hard" compounds. Adaptation experiments to A and B were carried out submitting the activated sludge population to succesive transfers in an artificial medium containing A and B as the sole carbon source. It was found (Fig. 6) that the degradation of A and B was increasing through the successive transfers and several species of bacteria adapted to grow an A and B were isolated. Degradation was followed measuring the COD according to the Standard Methods (1976).

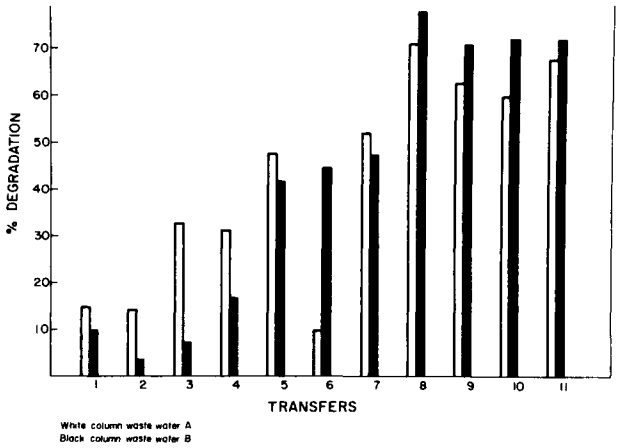

Fig. 6. Adaptation process.

Once the adaptation was achieved it was possible to establish some conditions to favour the development of ciliated protozoa and flocculating bacteria by addition of catalytic amounts of proteose-peptone-yeast extract to the activated sludge as a source of growth factors and vitamins. Experiments now in course suggest that the addition of low concentrations of 3-methyl benzoic acid (less than 100 ppm) also estimulates the development of flocculating bacteria and ciliated protozoa (Fig. 7-8). If the implantation of adapted populations to toxic substances into an activated sludge system is achieved the efficiency of the treatment plant would greatly improved.

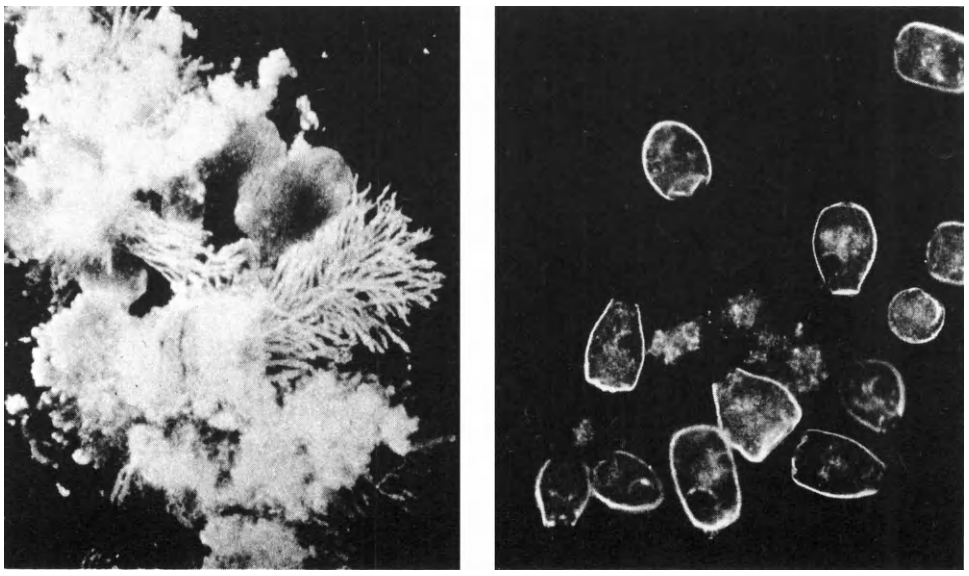

Fig. 7-8. Zoogloea ramigera and ciliated protozoa.

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The Surveillance of Coastal Marine Waters with Bivalves - The Mussel Watch

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ABSTRACT

Bivalves, oysters and mussels, have been used as sentinel organisms to monitor levels of heavy metals, petroleum hydrocarbons, synthetic organics, and artificial radionuclides at over 100 stations in the U. S. coastal waters. Zones of high pollutant levels have been identified. The strategies of carrying out the program, including collection techniques and analytical procedures, are described. The results from the initial analyses in 1976 and 1977 indicate varying degrees of pollution in the studied waters. Further, there is a remarkable similarity in the concentrations of some heavy metals and plutonium in the organisms collected from a given station during year one and during year two. In the future, other pollutants, such as those arising from the chlorination of waste waters, might be included in the program. Further, there is an urgent need for the development of methods to assess the biological impact of the pollutants, acting singly or in concert.

KEY WORDS

Bivalves, molluscs, oysters, artificial radionuclides, petroleum, synthetic organics, heavy metals, sentinel organisms.

INTRODUCTION

Coastal marine waters have been altered in many ways by the activities of human society. Substances toxic to living organisms have been deliberately or inadvertently introduced. The oceans have been littered with solid debris which can interfere with recreation, transportation, fishing or mining. Man-mobilized biostimulants have enhanced the growth of plants, oftimes unwanted ones, which compete with species forming the base of normal marine food chains. The aesthetic qualities of seawaters have been jeopardized by the accumulation of petroleum products. Many industrialized nations have recognized actual or potential losses of marine resources through the continued use of the oceans as waste space and have placed restrictions upon the discharge of substances identified as pollutants. Still, increasing uses of materials by an increasing world population are resulting in increasing burdens of foreign substances in coastal waters.

Records of changes in pollutant levels are essential for the effective managements of the coastal zone. They may be found in the waters themselves, in the sediments, or in the organisms inhabiting the waters. Each has both advantages and disadvantages.

The assays of waters give instantaneous exposure levels, the direct measurement of parameters that affect the vitality of resident organisms, and, as such, provide valuable information. However, the extremely low levels of many toxic substances make such analyses both time consuming and difficult and, in some cases, impossible.

The sediments contain environmental histories of those substances that are carried out of the waters on solid phases. In strata that accumulate under anoxic conditions or that rapidly become anoxic after deposition, bioturbation of the deposits can be low and the records can maintain their integrity. However, in deposits accumulating under oxidizing conditions, the records may be smeared by the burrowing and filtering activities of organisms. The sediments can often be sampled at annual intervals and thus the records give measures of pollutant levels averaged over a year or so.

The sentinel organisms are those that accumulate some pollutants at levels markedly enriched over seawater and as a function of the concentrations in the waters. Those organisms that as adults are attached to such objects as rocks or pilings or have restricted movements in the zone under survey are clearly preferable to those that migrate from place to place. Further, some bivalves, like the mussel Mytilus edulis, are rather widely distributed in the world's coastal waters and provide a common denominator for study.

Investigations over the past several decades have indicated that the bivalves are especially attractive for measuring exposure levels of a variety of pollutants (Goldberg, 1975). Artificially produced radionuclides, such as plutonium-238 and strontium-90, are enriched in both the shell and soft parts. Molluscs are well known concentrators of heavy metals. In both the United States and in northern Europe, they have been used in regional monitoring programs of halogenated hydrocarbons, such as the industrial chemicals, polychlorinated biphenyls, and as the persistent pesticides, DDT and dieldrin. They are useful as sentinels for petroleum hydrocarbon pollution. They rapidly take up both saturates and aromatics from their environmnet and store them with little metabolic breakdown. These abilities of bivalves circumvent the necessity to obtain large quantities of contaminant-free seawater and to transport them to laboratories for pollutant analysis. Thus, the organisms play a key role in analytical schemes by providing an initial enrichment step.

With this background, the U. S. Mussel Watch program was evolved under the aegis of the U. S. Environmental Protection Agency, Environmental Protection Laboratory, Kingston, Rhode Island and was coordinated by the Scripps Institution of Oceanography, University of California at San Diego, situated at La Jolla, California. Five participating laboratories were involved in the program: Scripps Institution of Oceanography, La Jolla, California; Woods Hole Oceanographic Institution, Woods Hole, Massachusetts; Moss Landing Marine Laboratory, Moss Landing, California; Marine Station, University of Texas, Port Aransas, Texas; and Bodega Marine Laboratory, University of

California, Bodega Bay, California. A guidance committee, which included the principal investigators from each of the laboratories, directed the program. The first results have recently been published (Goldberg et al., 1978). Herein we shall discuss the strategies of the program, the problems encountered and the initial results.

SAMPLING STRATEGIES

Three coastal areas of the U.S. were covered, the east coast, the west coast, and the gulf coast, with 107 stations. The samples locations are given in Figure 1. Samples were normally taken at a single site at each station, al-

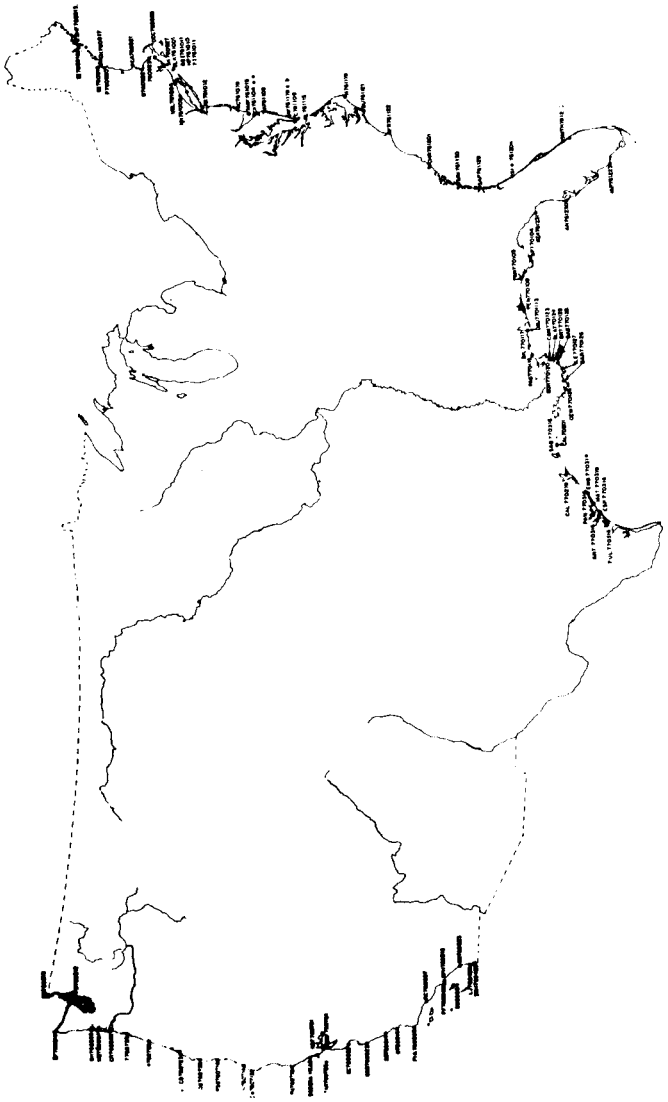

Figure 1. The sampling sites of the U. S. Mussel Watch

though on the west coast composite collections were made from up to eleven sub-stations at six of the sites.

The collections included the mussels Mytilus edulis and M. californianus and the oysters Crassostrea virginica and Ostrea equestris. Populations of large size were sampled such that only an insignificant number of its members were used. Animals of uniform size were sought, where possible approximately 5 to 8 centimeters long, although some oysters were somewhat larger.

Where possible samples were collected from rock, sand or mud environments. Pilings or metal buoys were avoided to minimize uptake by the bivalves of paints, creosote or other materials that might have been applied to them. Immediately after collection, the samples were placed in plastic bags (for heavy metal or radionuclide assay) or aluminum foil (for petroleum hydrocarbon and synthetic organic assay) and frozen. Samples were airshipped to the participating laboratories in styrofoam-lined cardboard shipping containers with dry ice.

A single scientist, operating from a camper, made the collections, from June to December, starting in Southern California and concluding with the Gulf Coast stations. This tactic provided uniformity in the types of organisms collected and in preservation techniques.

Collection costs in 1978 averaged about $1000 per station, including airshipments to laboratories and the maintenance of library specimens from all stations. Analytical costs were: Petroleum hydrocarbons and halogenated hydrocarbons, $1000 per sample; radionuclides, $550 per sample; Heavy metals $50 per sample.

ANALYTICAL TECHNIQUES

The pollutants studied are given in Table 1. There was a good deal of initial concern about establishing and maintaining both precision and accuracy in analyses. The participating laboratories had been analyzing the pollutants for many years before the initiation of the program and had

TABLE 1. Pollutants Analyzed in U. S. Mussel Watch

Heavy metals:	Lead, cadmium, silver, zinc, copper and nickel. Mercury was added in 1978.
Radionuclides:	Pu-239+240; Pu-238, Am-241 and Cs-137.
Halogenated hydrocarbons:	p, p' - DDE and p, p' - DDD PCBs (1254 and 1260).
Petroleum hydrocarbons:	Naphthalene, methylnaphthalenes, C-2 naphthalenes, C-3 naphthalenes, phenanthrene, methylphenanthrenes, C-2 phenanthrenes, fluoranthene, pyrene, chrysene/benzanthracene/triphenylene, benzopyrene/perylene/benzofluoroanthene, dibenzothiophene, and methyldibenzothiophene.

available primary standards, of their own devising or from other laboratories. It seemed reasonable to approach the problem by two participating laboratories analyzing a given collective of pollutants in monthly samples, which were collected from Narragansett Bay, Rhode Island, and from Bodega Head, California. In such a way, the differences in sample treatment, analytical technique, or in methods of calculation might be revealed through systematic differences in the results from the two laboratories.

Intercalibration studies for heavy metals were carried out by the Moss Landing Marine Laboratory and the Scripps Institution of Oceanography. Satisfactory agreement was reached for the zinc, copper, silver, cadmium and nickel assays. Higher levels of cadmium were systematically found by the La Jolla laboratory, while lower levels for lead were observed (Table 2). These differences may arise from variations in sample treatment (La Jolla dries samples at 110° while Moss Landing freeze-dries the soft parts), from real differences in the composition of the analyzed mussels, even though taken from the same site, or from slight inaccuracies perpetrated by one or both laboratories.

TABLE 2. Heavy Metal Intercomparison Analyses of Mytilus Californianus. Assays of Monthly Samples from Bodega Head. Results in Parts Per Million of Dry Weight. LJ = La Jolla; ML = Moss Landing.

Sample No.	Lab.	Pb	Cd	Ag	Zn	Cu	Ni
760324	LJ	1.0	11.2	0.2	130	7.9	3.9
	ML	1.0	9.5	0.1	140	6.4	1.8
760910	LJ	0.6	12.4	0.05	115	4.9	1.5
	ML	2.7	7.8	0.2	100	5.9	2.7
77104	LJ	0.8	9.9	0.18	135	6.3	1.7
	ML	2.9	7.2	0.4	130	6.9	2.5
770202	LJ	0.6	9.1	0.38	110	6.4	1.7
	ML	3.3	6.9	0.3	110	8.0	4.1

A similar exercise was carried out for the radionuclides by the La Jolla and Woods Hole Institution of Oceanography workers. Both laboratories treated the samples somewhat differently. The Woods Hole laboratory dried their samples at 150°C, while the La Jolla Investigators used a temperature of 110°C. The Woods Hole group reported their data on a wet weight basis, while the La Jolla laboratory utilized the dry weight basis. However, the conversion from wet weight to dry weight can readily be made by using a wet to dry weight ratio of 7.5. The La Jolla values were converted to a wet weight basis, using wet weight measurements made in their laboratory. Some of the variations in the results (Table 3) could arise from losses of water in handling the samples as well as in real differences in the water contents of two samples from the same collection. The agreement in the results from the two laboratories are satisfying for these very low levels of radioactivity.

TABLE 3. Intercalibration studies on radionuclides in *Mytilus californianus* by the La Jolla and the Woods Hole Laboratories. LJ = La Jolla. WHOI = Woods Hole.

Sample	Laboratory	Wet weight / Dry weight	Pu-239+240	Am-240	Cs-137
			in dpm/kilogram wet weight		
BH 760324	WHOI	6.3	0.92	2.2	3.6
	LJ	9.1	0.46	1.1	
BH 760729	WHOI	6.6	0.58	1.7	2.5
	LJ	8.3	0.34	0.8	5.3
BH 761202	WHOI	7.7	0.22	0.96	3.7
	LJ	8.1	0.25	0.98	

The Woods Hole and Bodega Head laboratories assayed the degradation products of DDT, pp'-DDE and p,p'-DDD, as well as PCB 1254 in the monthly samples. A systematic difference in the pesticide assays appears, whereas there is close agreement on the PCB 1254 analyses. The results from Woods Hole are about twice as high for DDE and substantially less for DDD in the three comparison samples (Table 4). Due to the low concentrations of the DDT family members in this intercalibration study, these differences are not viewed with concern and do not influence the interpretation of the data. Still, the variations in the analyses are being investigated via further intercalibration studies.

TABLE 4. Intercalibration analyses of Bodega Head Mussels (*Mytilus californianus*) for Halogenated Hydrocarbon concentrations by the Bodega Marine Laboratory (BML) and the Woods Hole Oceanographic Institution (WHOI).

Sample No.	DDE	DDD	PCB	
	in units of nannograms/gram dry-weight			
BH 760324	17	8	--	BML
	34	--	--	WHOI
BH 760603	10	13	--	BML
	20	--	--	WHOI
BH 760729	23	12	--	BML
	14	--	--	WHOI
BH 761605	7	10	18	BML
	14	1.3	18	WHOI
BH 761202	17	8	18	BML
	35	2.7	21	WHOI
BH 770104	10	39	19	BML
	18	3.3	17	WHOI

Inasmuch as one of the laboratories analyzing the petroleum hydrocarbons has as yet to report its first results, intercomparison studies between two groups cannot be made.

LIBRARY OF MUSSEL SAMPLES

About one kilogram of mussels from each of the collection sites has been frozen and incorporated into a library at the Scripps Institution of Oceanography. This library will be especially advantageous for the determination of pollutants not currently being analyzed but identified at some future time. It is recognized that some of the pollutants may undergo degradation at the freezer temperature of about -10°C. Radioactive substances decay; organic molecules may be decomposed by bacteria or abiotically. While heavy metals, long-lived radioactive species and organic substances may be translocated in the library samples, they will still respond to analytical techniques at some later date.

BIBLIOGRAPHY OF POLLUTANTS IN BIVALVES

An initial literature search yielded about 500 references to pollutant levels in bivalves. The articles have been abstracted into a single volume in which the pollutants analyzed, techniques used, organisms studied, the times and locations of collection and the analytical results are presented. The volume, hopefully, will be continually updated with new or additional citations. The first edition was published in November 1977 and was distributed to the program participants and other interested scientists.

IDENTIFICATION OF PRIORITY RESEARCH AREAS

During the course of the work during the first two years, it became evident that there were research activities needed to implement and strengthen the monitoring project. Some of these are now under active study; others remain to be initiated.

1. In the analyses of synthetic organic compounds by variations of gas chromatographic techniques, the spectra contain many unidentified peaks, some of which may be those of halogenated hydrocarbon pollutants. The identification of such peaks is both time consuming and expensive. Perhaps, the most reasonable tactic is a computer storage of the spectra by a program used by all investigators. Where identification of a peak is made at a future time and an association with a man-made substance is established, the computer can be questioned about its concentrations in the samples analyzed previously. Where an association of a peak with a geographical location or a point in time is unique, an effort can be made to identify it.

2. The contents, as opposed to concentrations, of some metals appear to be a function of size and age, yet simple techniques for ascertaining the ages of bivalves from the field are yet to be established. The concentrations of metals in bivalves can increase, decrease or remain unchanged with age (Boyden, 1977). Thus, the age distributions of the organisms analyzed are important for interpretation of the results.

Age determinations are usually based upon the counting of annual rings in the shells or by shell dimensions. Recently, Griffin et al (1978) have proposed two novel methods. One involves measurement of the valve thickness in units of mg/cm^2. The other involves the contents of Pb-210, Po-210, or Pb in the soft parts of the mussels. All of the methods give concordant ages for mussels collected off the California coast.

3. One possibility for effectively and efficiently using bivalves in monitoring schemes involves the use of shell concentrations. It seems reasonable that the biological half-lives in the shells would differ from those in the soft parts in the direction of being longer. The covariance between Pu-239+240 concentrations in the soft parts and in the shells for mussels from the western U.S. coast is shown in Figure 2. For these same specimens the Am-241/Pu-239+240 ratios in the shells were usually within a factor of two of those in the soft parts. Griffin et al (1978) have shown that there is a strong covariance between the logarithm of the Po-210 contents of the shells with the mussel ages as measured by valve thickness. A similar covariance exists for the Po-210 contents in the soft tissue (Figure 3). The potential use of the shell for the record keeping of environmental levels of pollutants appears promising. Clearly, the shell and the soft parts will integrate over different time periods for each pollutant, as a function of the biological half-lives in each of these domains. This is an area where more extensive researches are needed, not only with respect to metals, but with respect to the other pollutants, such as the petroleum hydrocarbons and synthetic organics.

INITIAL RESULTS OF THE U.S. MUSSEL WATCH PROGRAM

The bivalve analyses have indicated varying degrees of pollution in some of the over hundred localities sampled in the United States. High levels of DDT and its degradation products were found in mussels taken from San Francisco, California and San Diego, California. The source of these pesticides is a manufacturing plant in Los Angeles which discharged its waste through a Los Angeles sewer outfall. In 1971, for example, 19 metric tons of DDT residues entered the waters off Los Angeles from the plant. The plant switched to a sanitary landfill disposal operation in the early 1970s and the present day fluxes arise from residual materials in the sewer pipes or in the sediments.

On the other hand, inputs from multiple sources are probably responsible for the high levels of polychlorinated biphenyls in mussels from San Francisco Bay, San Pedro Harbor and the New York-Boston coastal zone. Values two to three orders of magnitude greater than background concentrations are found in mussels living in waters situated adjacent to sites of high industrial activity. Perhaps the highest PCB contents in mussels yet found are in those from New Bedford, Massachusetts. Here the source may be a manufacturer of electrical capacitors, leakages from whose plants may have taken place over the last several decades.

There are a number of heavy-metal "hot-spots". Elevated levels appear in mussels from the New York-New Haven area. In the east coast samples, the highest concentrations of copper and cadmium occur in the organisms collected at New Haven Harbor, Connecticut. Whether these analyses reveal natural estuarine levels or contaminant effects is as yet unknown. On the Pacific and Gulf coasts there were no obvious areas of metal pollution as revealed by mussel analyses.

Of the 83 samples from the Gulf and Pacific Coasts analyzed during the first year for petroleum pollution, only four gave positive results. These mussels contained aromatic compounds characteristic of petroleums, not found as natural constituents of living matter. In the case of the four polluted samples, the benzene eluate contained the petroleum-derived aromatic hydrocarbons

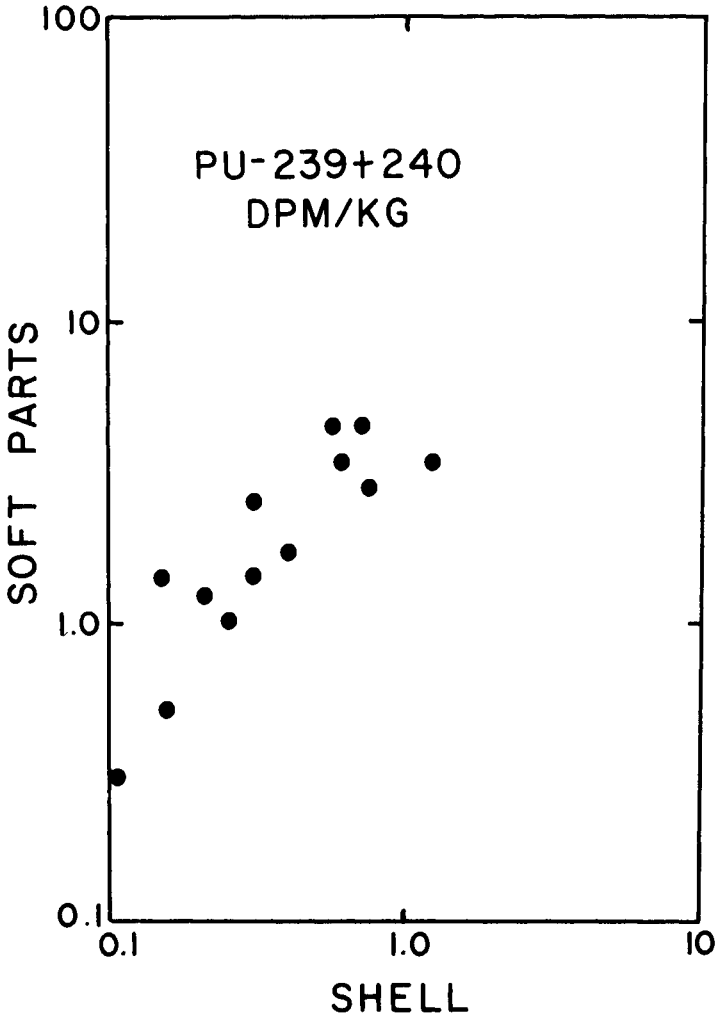

Figure 2. Plutonium 239+240 in the soft parts and in the shells of Mytilus edulis.

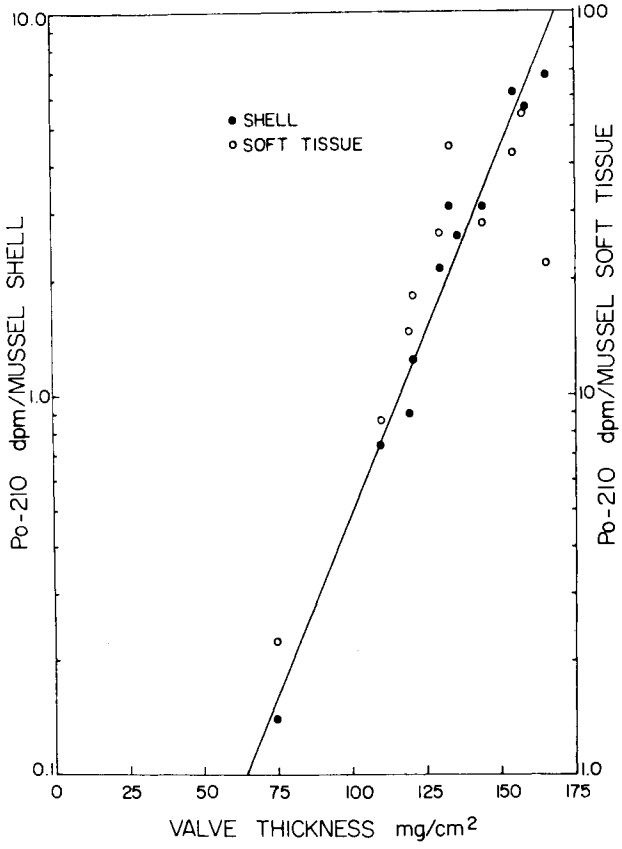

Figure 3. Po-210 in the shells and soft tissues of M. edulis. The valve thickness is a measure of age with each 100 mg/cm^2 corresponding to one year.

phenanthrene, C_1-C_3 substituted alkyl phenanthrenes, and C_1-C_3 substituted alkyl dibenzothiophenes. In the one west coast sample that showed petroleum pollution, Boundary Bay, Washington, the hexane eluate contained a full of petroleum-like n-paraffins, as well as the petroleum-indicator compounds listed above.

The heavy metal and plutonium data for the first two years of the program, 1976 and 1977, are plotted in Fig. 4. There is a remarkable co-variance for the concentrations in the first and second years, extending over two orders of magnitude for zinc and lead and one order of magnitude for plutonium and cadmium. For the metals, oyster and mussel concentrations are plotted, which accounts for some of the observed wide ranges of values. Either or both of two factors may govern this phenomenon. First of all, the metals may have long half-lives in the organisms, of the order of years, similar to those of Pb, Pb-210 and Po-210 (Griffin et al., 1978). Or, the environmental concentrations of these elements may have been uniform over the period of analysis i.e. these elements have residence times in the environment of the order of years.

Irregardless of the factors which have governed the similarity in concentrations for these two years, the data suggest that yearly sampling, at least for these elements, may not be necessary. The immediate goal of the Mussel Watch Program is to assess pollutant levels and their changes with time in a given coastal marine zone. In order to identify significant changes in concentration of these elements, a sampling period of the order of several years may be more appropriate and economic.

Since sampling from a given site was only made once a year, there was a concern that the concentrations of a pollutant might show seasonal trends as a function of food supply, sexual state, water characteristics such as salinity, etc. No significant seasonal variations of heavy metal or halogenated hydrocarbon concentrations were observed in the monthly samples taken at Narragansett Bay or at Bodega Head. The lack of a seasonal effect could result from long biological half-lives of the pollutants in the organisms and/or from uniform levels of the pollutants in the seawater or in their food. Some recent studies point to the former explanation, at least in the case of the heavy metals.

The biological half-lives of Pb, Pb-210 and Po-210, have been estimated to be of the order of several years on the basis of their contents in samples collected from the field (Griffin, et al., 1978).

Boyden (Boyden, 1977) has demonstrated that many heavy metals show increasing contents in bivalves with increasing size, whereas the concentrations are either uniform or possess modest positive or negative regressions with size. Thus, the collection of similar sized organisms from a given area, where the environmental levels have not changed with time, should result in the situation where sampling at any time during the year gives a value representative of the area.

Unlike the heavy metals, there were changes in the radionuclide concentrations in the mussels with time. At Narragansett, the Pu levels were uniform in June, July and August of 1976 but subsequently declined by a factor of two. Am-241 showed similar changes. At Bodega Head, the March-June concentrations were similar but declined slowly up through October-December.

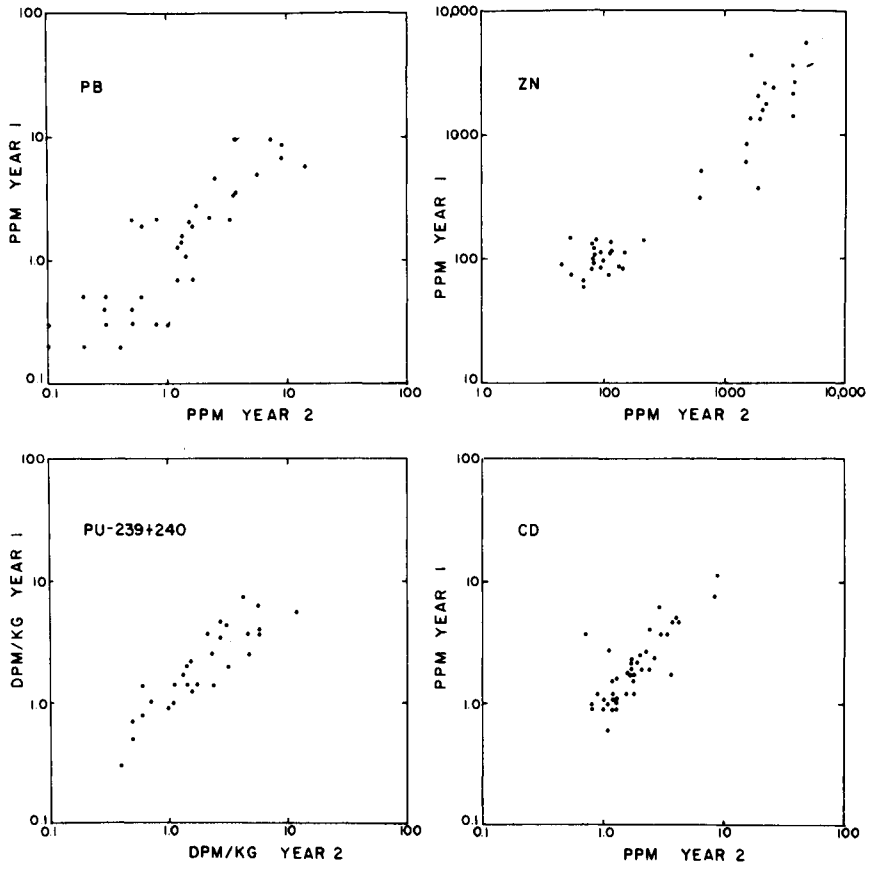

Figure 4. The heavy metals and plutonium in the soft tissues of mussels collected from various locations during year one and year two of the U. S. program.

Since no comparable changes were found with the heavy metals, as might be predicted if there were changes in the mussel mineral physiology, the radionuclide changes were attributed to changes in their supply or availability, i.e. environmental levels. Further, the results suggest that the biological half-lives of those transuranics are shorter than those, say, of lead and polonium.

FUTURE DIRECTIONS

There are several general areas of activity that might be pursued to implement and expand the use of bivalves as sentinels of the health of coastal waters. First of all, they may maintain records of other sets of pollutants that have been identified. For example, the use of chlorine (and ozone) in water treatment process is producing in sea water a group of chlorinated and brominated compounds that are biologically active. These toxic chemicals include chlorphenols and chlorinated pyrimidines; the majority of halogenated compounds arising from these oxidations are as vet unidentified. The sources are the discharges of waste and cooling waters from municipal treatment plants and from electric power generating plants. Bivalves in suitable locations near these plants may serve as effective monitors.

Perhaps the bivalves through their filter feeding activities could be used to monitor the micro-organisms of coastal waters, especially those which are agents of human or faunal disease.

The health of the bivalves may provide a measurement of pollutant levels in the environment. In the first report of the Mussel Watch (Goldberg, et al. 1978) it was indicated that on the basis of histopathological studies there are evidences of frequent occurrences of unhealthy mussels. Thirty nine mussels were examined and over one third (15) were found to be in poor or very poor condition, primarily as a result of parasitic infestation. Degradation of visceral components was observed. The bivalves may have been subjected to physiological stresses by the pollutants. This possibility should be investigated through laboratory and field exposure of bivalves to pollutants, either singly or in combination.

The determination of biological impacts by pollutants, acting either singly or in concert, still eludes us. Although the proper functioning of marine organisms can be affected by man's mobilization of chemicals, be it involving behavioral, respiratory, reproductive or other metabolic processes, causal relationships with pollutants are yet to be established for ready use in assessing the viability of ocean waters. Some scientists suggest that studies of community composition and perturbations upon them by pollutants may provide the key to biological impacts. Such investigations would require years of work to establish the normal composition of a community and to determine what are the ranges of variation of its members. These long term studies might be carried out at a variety of coastal sites. Still, the challenge of the problem is attracting more and more workers to its fold and hopefully solutions will become available within a reasonable time period.

ACKNOWLEDGMENT

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Environmental Dynamics of Trace Organics Contaminants in Estuarine and Coastal Zone Ecosystems

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ABSTRACT

The distribution and bioaccumulation characteristics of man made trace organic compounds in the marine environment are discussed in terms of the physical/chemical processes that control their flow throughout the ecosystem. For the low levels detected in seawater, the data suggest that biological uptake is predominantly governed by equilibrium partitioning of the chemicals between biota and ambient water. Theoretical computations of distribution coefficients between water and suspended phases for certain contaminants have been validated. The use of these parameters in environmental impact and criteria requirements for open water industrial and municipal discharges as well as dredge disposal operations are presented. The feasibility for using this approach in formulating long-term monitoring strategies for the Mediterranean Sea in view of the existing level of contamination is also discussed.

INTRODUCTION

When an organic compound is introduced into a marine ecosystem, it interacts with the abiotic and biotic components of the system. Considering the extreme complexity of these processes, it seems unlikely that the flow mechanism can be evaluated from fundamental physical and biochemical considerations within a reasonable time frame. Consequently, it is desirable to seek empirical parameters that: can be easily measured in the aquatic environment; reflect net effects of complex transport and chemical transformation steps and; can be used as universal indices in predicting the distribution of chemicals within marine components.

THEORETICAL CONSIDERATIONS

A parameter that appears to meet these criteria is the distribution coefficient, K, defined as: $K_X = \frac{C_{x(j)}}{C_{x(i)}}$ where $C_{x(j)}$ is the concentration of chemical x in component j and $C_{x(i)}$ is the corresponding concentration of x in component i.

Since interactions for persistent organic chemicals are limited to intercomponent transfers, K for these compounds represents the instantaneous distribution or partitioning of x between the marine components in question. With rapid exchange rates K becomes a quasiequilibrium constant for the transfer reactions and can be derived from theory.

Suspended Particulate Matter (SPM) and Sediments

The distribution of non-polar organic compounds at the sediment-seawater interface can be represented by an equilibrium sorption mechanism. A general theoretical expression of the distribution coefficient, K, has been developed based on a simple BET isotherm and assuming 1) London dispersive and hydrophobic interactions are the predominate factors influencing sorption and 2) the system is equilibrated at low ambient concentrations of the chemical (<5% saturation). The general form of the expression is:

$$K = \frac{C_{X(S)}}{C_{X(W)}} = \frac{A_S^* M_X \exp[(E_S - E_m)/RT]}{\bar{A}_X C^o_{X(W)}} \qquad (1)$$

where A_S^* is the specific surface area of the ambient SPM (cm^2/gram), $\bar{A}$ denotes the molar area of x (cm^2/mole), M_X is the molecular weight of the adsorbate (g/mole) and $C^o_{X(W)}$ refers to the solubility of the adsorbate (g/g). $C_{X(S)}$ and $C_{X(W)}$ correspond to the concentration of adsorbate on SPM and in solution (g/g), respectively. E_S is the energy of interaction of x with the bare surface (formation of the first adsorption layer) and E_m is the energy of interaction with the covered surface (formation of multilayers); both quantities are in units of cal/mol. R refers to the gas constant (cal/deg/mole) and T to the temperature (deg).

Equation (1) has been further modified to account for variations in natural particle sizes and content of organic matter, since there is evidence that these variables contribute significantly in the marine environment.

The expanded version of equation (1) is then:

$$K = \frac{6\alpha M_X[1-\exp(-A_p^* P d_S \rho_S/6\alpha)] \exp[(E_S - E_m)/RT]}{d_S \rho_S \bar{A}_X C^o_{X(W)}} \qquad (2)$$

Where A_p^* is the surface area occupied by a unit mass of natural organic matter P(cm^2/gm); P is the fractional organic matter content; α is a constant to account for increased surface area beyond that specified for spherical particles; d_S refers to the effective diameter of the particle (cm); and ρ_S is the particle density (gm/cm^3).

Both expressions have been used in estimating the distribution coefficients for a number of molecules in fresh water and marine systems. Based on equation (1), plots of $C_{X(S)}$ and $C_{X(W)}$ for tetrachlorobiphenyl are shown in Figure 1. The isotherms for three values of $(E_S - E_m)$ are plotted. Figure 2 is a blow up of the low region of the curve with data superimposed on it.

The dependence of K on particle size and organic content is depicted in Figure 3. The plots represent the predicted behavior for kepone on various particle

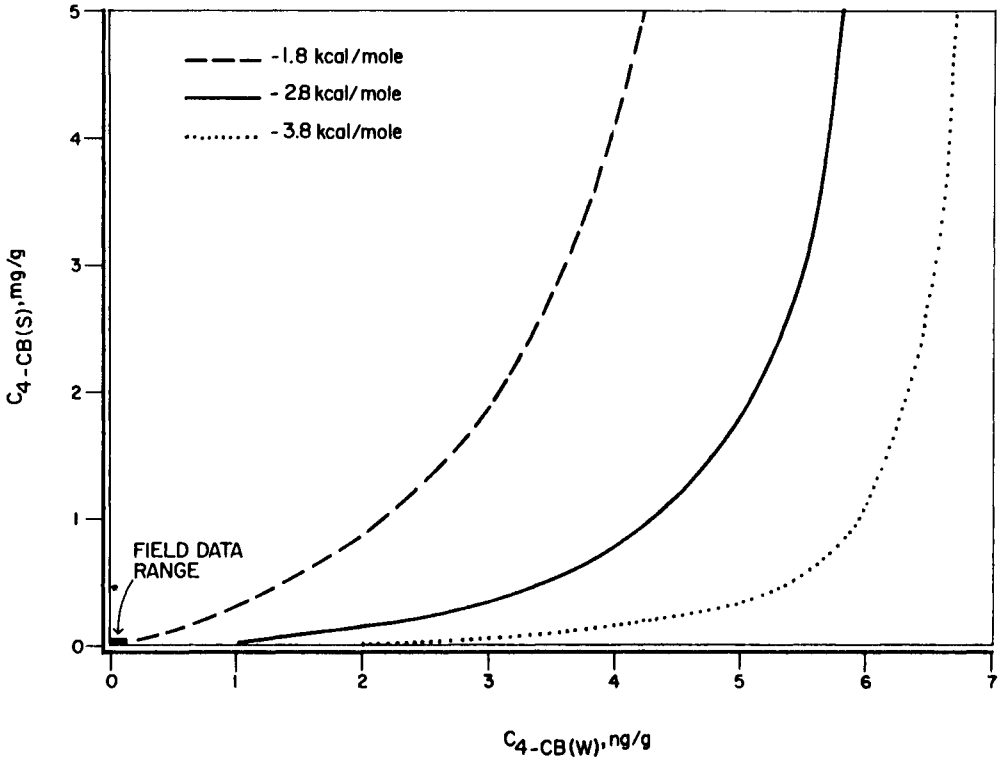

FIGURE 1 Plots of the predicted concentration of tetrachlorobiphenlys on the particle surface, $C_{4\text{-}CB(s)}$, versus the aqueous concentration, $C_{4\text{-}CB(w)}$, for three adsorption energy values: solid line -2.8 kcal $mole^{-1}$; dashed line -1.8 kcal $mole^{-1}$; dotted line, -3.8 kcal mole. The small blackened portion of the plot indicates the maximum range of the observed field data.

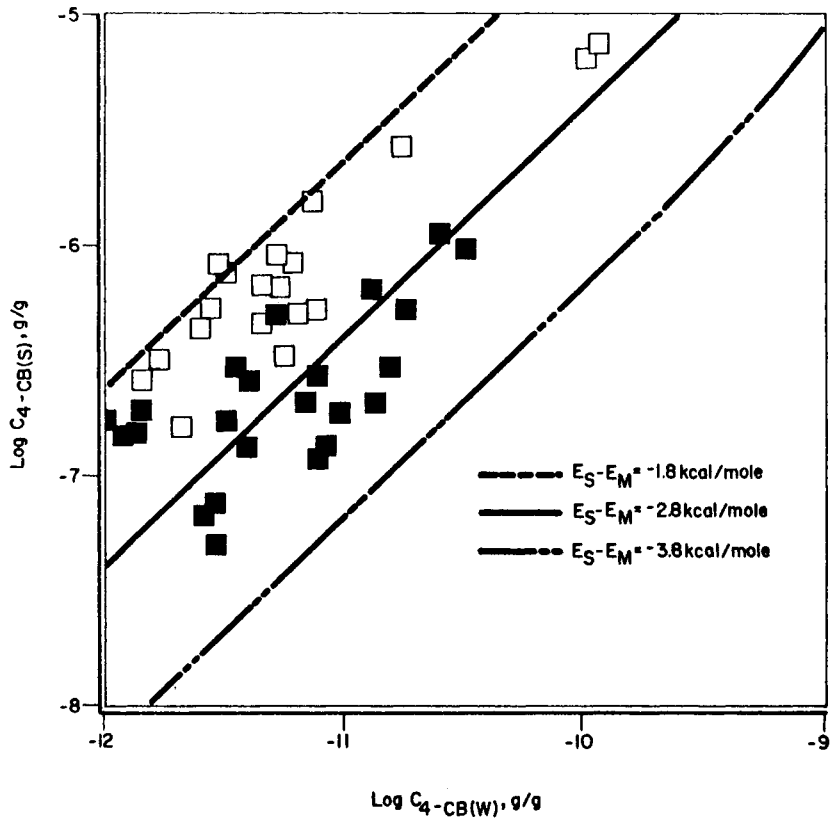

FIGURE 2 Plots of the logorithm of the surface concentration of tetrachlorobiphenyls, $C_{4\text{-CB(s)}}$, versus the logorithm of the aqueous concentration, $C_{4\text{-CB(w)}}$. The solid lines represent the predicted relationship for the energy levels of Figure 1. Symbols are the observed field data: , values from the Duwamish River, March 1976; , values from Puget Sound 1973-1976.

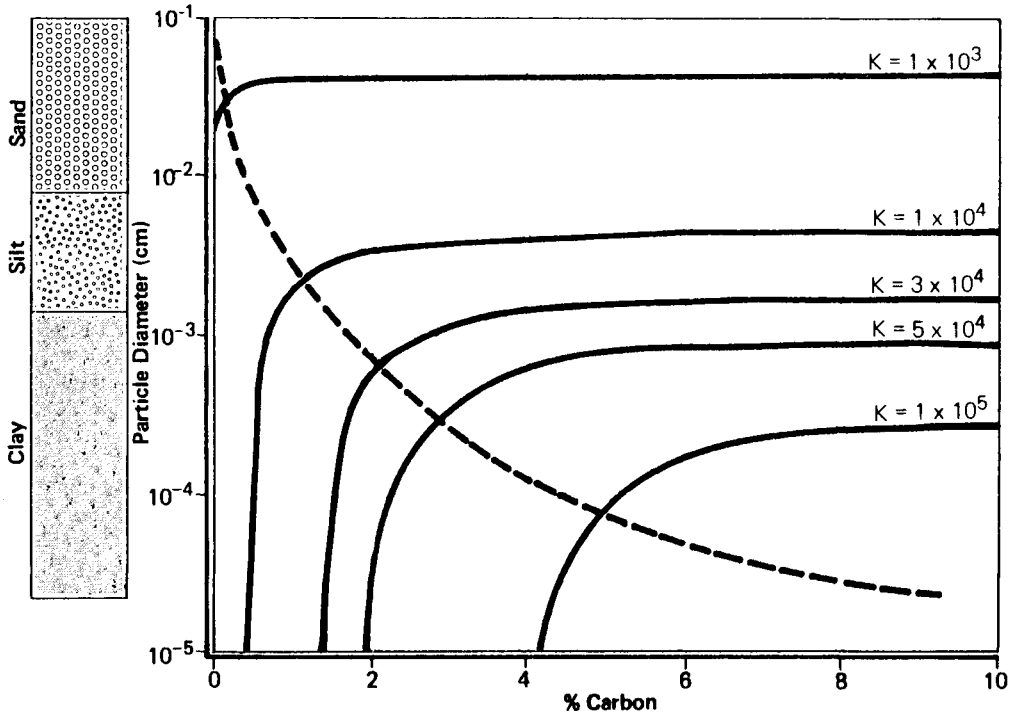

FIGURE 3 Plots of the distribution coefficient K, as a function of particle diameter and percent carbon for Kepone as predicted from equation (2).

sizes at different organic loadings.

Biota. The applicability of the equilibrium partitioning concept to predict accumulation of persistent chemicals in zooplankton has been also examined. For chlorinated hydrocarbons bioaccumulation is predominantly controlled by equilibrium partitioning between the internal lipid pools of the biota and ambient water. Amplification factors of 10^6 have been determined in terms of lipid water distribution coefficients. These quantities were uniform over a wide range of spatial and temporal regimes, species composition and lipid content. It may therefore be proposed that at least for pelagic biota at the lower trophic levels, food chain biomagnification was not a controlling factor in attaining the residue levels measured. A summary version of the information generated in these studies is presented in Table 1. Application of the theoretical model derived for suspended matter and sediments has not been attempted for zooplankton since a simple sorption isotherm does not represent the complex lipid transfer steps involved in the uptake process. However, it should be pointed out here that for organisms with low lipid content (<2%) the K values approach those determined for suspended particulates and sediments.

PREDICTION OF ECOSYSTEMS DISTRIBUTION

The utility of K in the marine environment is not limited only to the prediction of the accumulation potential of an organic compound. If it can be ascertained that K is constant within an ecosystem, then the fractionation of an organic chemical between two marine components can be expressed in a more useful form. Considering the fractionation of the residues of molecule x between water and phase j, the absolute amount of x within a volume of water, $C_{t(w),v}$, can be expressed as:

$$C_{t(w),v} = C_{x(w)} + \sum_j C_{x(j),v} \qquad (3)$$

where $C_{x(w)}$ is the concentration in water; and $C_{x(j),v}$ refers to the amount bound to phase j per unit volume of water. By definition:

$$C_{x(j),v} = C_{x(j),d} m_{j(w),v} \qquad (4)$$

where $C_{x(j),d}$ is the concentration of x per dry mass of j and $m_{j(w),v}$ is the dry mass of j per unit volume of water. By combining these two equations and Equation 1, the ratio of x bound to phase j to its total amount in water can be obtained as:

$$F = \frac{C_{x(j),v}}{C_{t(w),v}} = \frac{K_x m_{j(w),v}}{K_x m_{j(w),v} + 1} \qquad (5)$$

i.e., Equation 5 states that the fraction associated with component j will be a function only of the magnitude K_x and the ambient concentration of j. We have applied this treatment to examine the distribution of polychlorinated biphenyls (PCB) in estuaries and coastal zones. Figure 4 shows plots of F (expressed in percent) vs. $m_{j(w),v}$ for a number of K values superimposed on field and laboratory data for SPM and zooplankton. A good agreement with the predicted behavior is shown for SPM and phytoplankton. Although the K values for

TABLE 1 Summary of the Regional Mean K_1^N Values for Zooplankton and Sea Water in Puget Sound

REGION	n	$K_1^N \times 10^6$ N = 4	N = 5	N = 6	DOMINANT FAUNA
Elliott Bay	6	1.06 (±0.81)	1.42 (±1.06)	2.17 (±1.64)	Euphasiids
Main Basin	4	1.07 (±0.61)	1.90 (±1.25)	3.18 (±2.32)	Copepods
Whidbey Basin (Port Gardner)	12	0.80 (±0.38)	1.09 (±0.51)	1.47 (±0.75)	Copepods/Euphasiids
Hood Canal	3	0.98 (±0.56)	0.74 (±0.52)	0.43 (±0.32)	Ctenophores
Sinclair Inlet	3	1.12 (±0.68)	3.61 (±2.02)	6.90 (±3.45)	Ctenophores
Admiralty Inlet and Straits of Juan de Fuca	3	0.34 (±0.21)	0.28 (±0.18)	0.29 (±0.18)	Copepods
Average*		0.90	1.51	2.41	

*The unweighted means of all regional values.

Adopted from J.R. Clayton Jr., S.P. Pavlou, and N.F. Breitner
Environmental Science and Technology, 11: 676, 1977

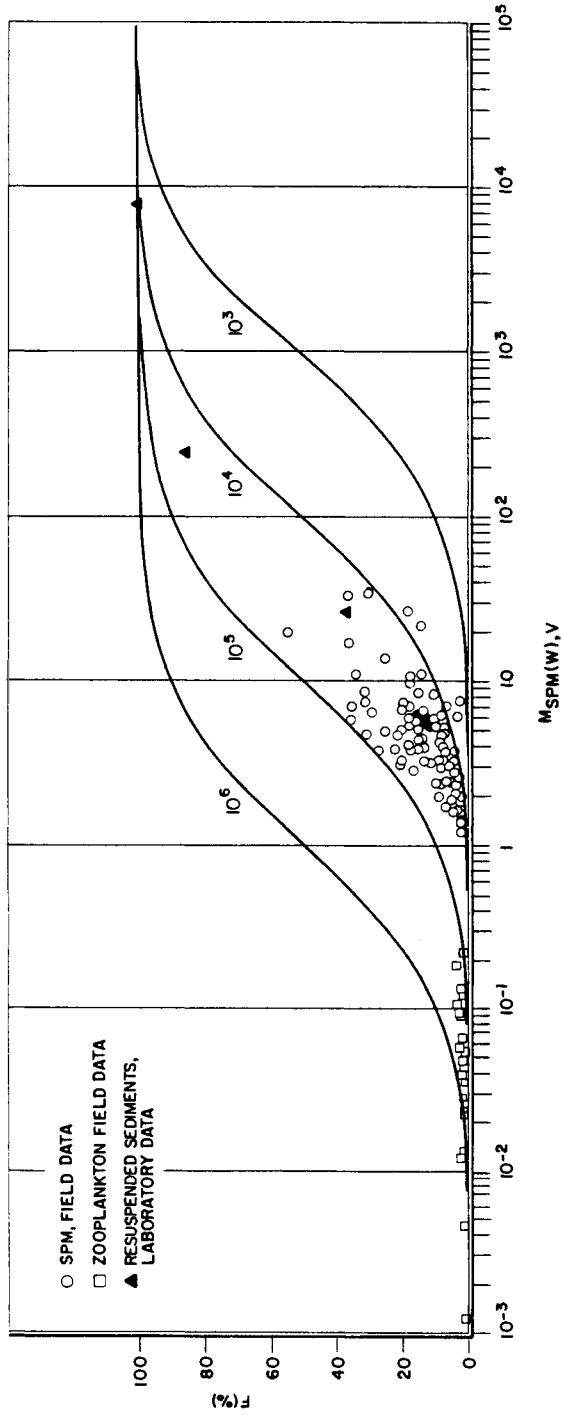

FIGURE 4 Plots of the sorption ratio, F versus the ambient suspended load, $m_{J(W),V}$. The solid lines represent the predicted behavior of F for a number of K values as computed from equation (5). The data represent field and laboratory measurements.

zooplankton were greater than the ones for SPM, the dry mass concentrations of the former were usually at least an order of magnitude lower than the corresponding SPM values. As a result, F for zooplankton is usually small; for these studies it never exceeded 4%. For the upper trophic level biota one can use the same argument, i.e., these systems will exhibit even a lower F value considering that there is a reduction of biomass in each succeeding trophic level, normally of about an order of magnitude for each level, but with no commensurate increase in the K values. These observations have some important connotations regarding the way the PCBs and other similar organic compounds fractionate between water and suspended phases and between water and sediments.

Since the normal range for the suspended load encountered in coastal zones is within 0.5 to 10 g/m^3, compounds with $K \leq 1 \times 10^5$ will reside primarily in the water and not on suspended phases. For example, the PCBs, which have K values within the range of 1×10^4 to 1×10^5, show an accumulation on suspended phases of less than 20% of the total PCB load in the water. Therefore, the spatial distribution of these chemicals will depend mainly on the hydrodynamics of the water column. Regarding their partitioning between water and sediments, it should be noted that the dry mass of natural sediments is normally about 50% ($\simeq 5 \times 10^5$ g/m^3) of their wet weight at the solid/water interface. Therefore, F should approach unity; this is clearly demonstrated in data obtained with resuspended sediments as shown in Figure 4.

It is also expected that under equilibrium conditions the PCB concentrations in sediments of a system receiving relatively uniform input should not vary greatly from the corresponding SPM concentrations in the overlying water. This assertion is supported by the data shown in Table 2. The agreement between the PCB sediment concentrations and those for the SPM collected at the same stations is good.

This correspondence is important from a monitoring standpoint, since the concentrations of PCB in the water and other ecosystem components can be inferred from the levels observed in the sediments. The latter are relatively easy to sample and analyze.

Based on the above considerations, the spatial distribution of persistent organic residues in the surface sediments should reflect the long term, integrated flow patterns of the overlying water. This argument is supported by the distributions of PCBs observed in Puget Sound, as well as in other coastal regions. A detailed PCB sediment map of Elliott Bay is shown in Figure 5 and confirms the Duwamish River as a source. The spatial patterns agree well with the prevailing circulation. Similarly, contours of the concentrations of other chlorinated hydrocarbons observed on the Palos Verdes shelf near Los Angeles (Figure 6) show patterns which clearly reflect the predominant northerly longshore water mass transport in the area. In some regions of the Eastern Mediterranean a similar behavior in the deposition of PCB has been observed (Figure 7). These observations therefore give evidence to the applicability of Equation 5 and the validity of the equilibrium partitioning concept.

The results from these studies support the suggestion that the sediments can provide a rapid assessment of the mean residue levels and dynamics of persistent organic chemicals, since on a mass basis they will always contain higher concentrations of residues and can easily be sampled and analyzed. However, it should be emphasized that such data must be interpreted with care. Even though the levels in the sediments represent temporal mean concentrations, they

depend also on other factors, such as the sedimentation rate in the area, the depth of sediment taken for analysis, the particle size, and the natural organic matter content.

The temporal and spatial uniformity of the distribution coefficients computed for suspended phases in the various marine regions examined provide supporting information on the validity of equilibrium partitioning and the applicability of the K quantities as universal indices of potential bioaccumulation.

TABLE 2 Comparisons of the Total Chlorobiphenyl Concentrations in Surface Sediments and SPM from Some Regions of Puget Sound[a]

REGION	SEDIMENTS (ng/g)	SPM (ng/g)
Duwamish River	1240 ± 251 (14)	1005 ± 284 (21)
Elliott Bay	164 ± 39 (9)	173 ± 24 (13)
Main Basin	70 ± 9 (3)	82 ± 3 (3)
Whidbey Basin	63 ± 16 (5)	82 ± 37 (7)

[a]Values in parentheses represent the number of data points.

Adopted from S.P. Pavlou and R.N. Dexter,
Environmental Science and Technology, In Press, 1978

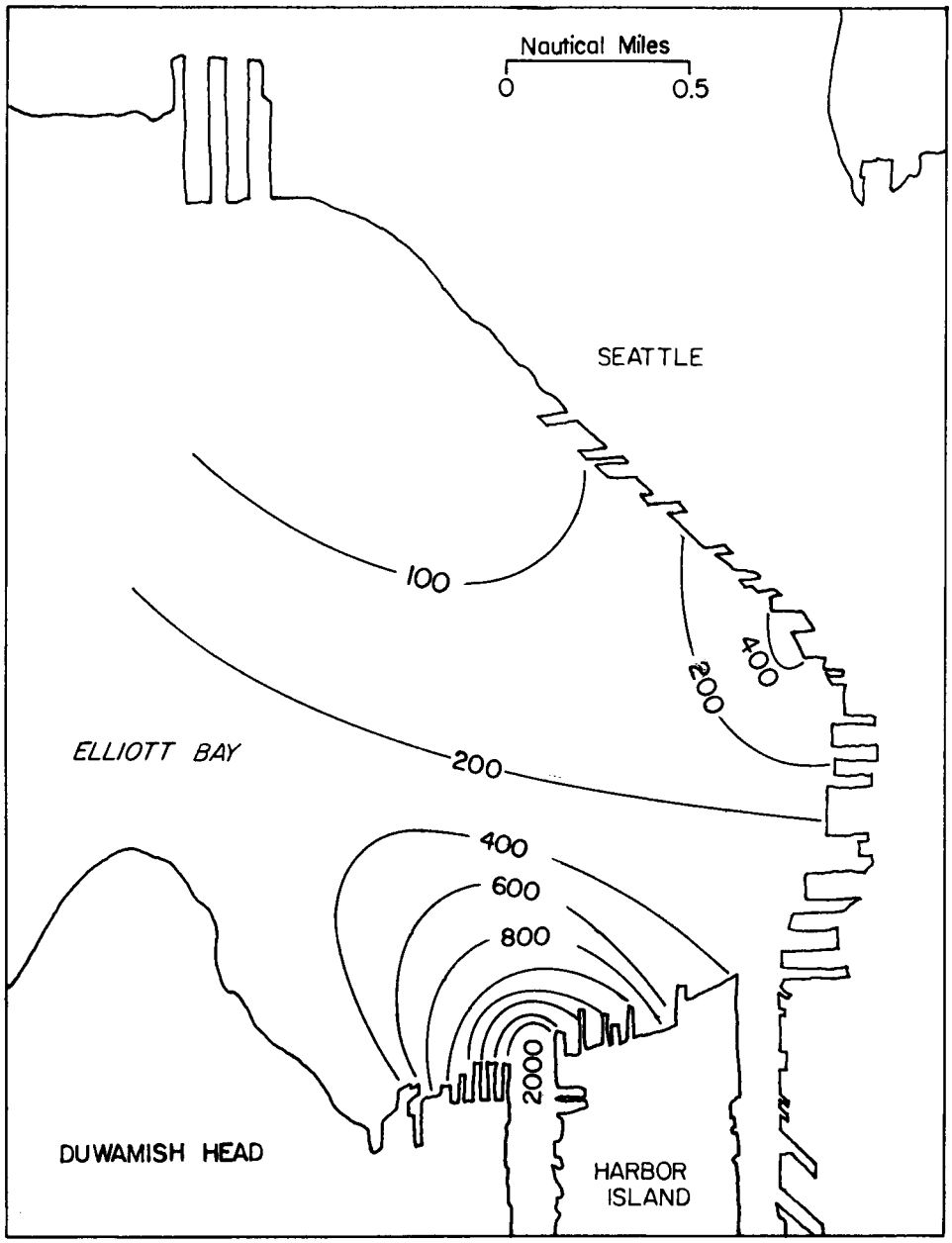

FIGURE 5 Spatial distribution of PCB's in surface sediments observed in Elliott Bay, Seattle, Washington. Concentrations are in units of mg PCB/kg Dry Sediments.

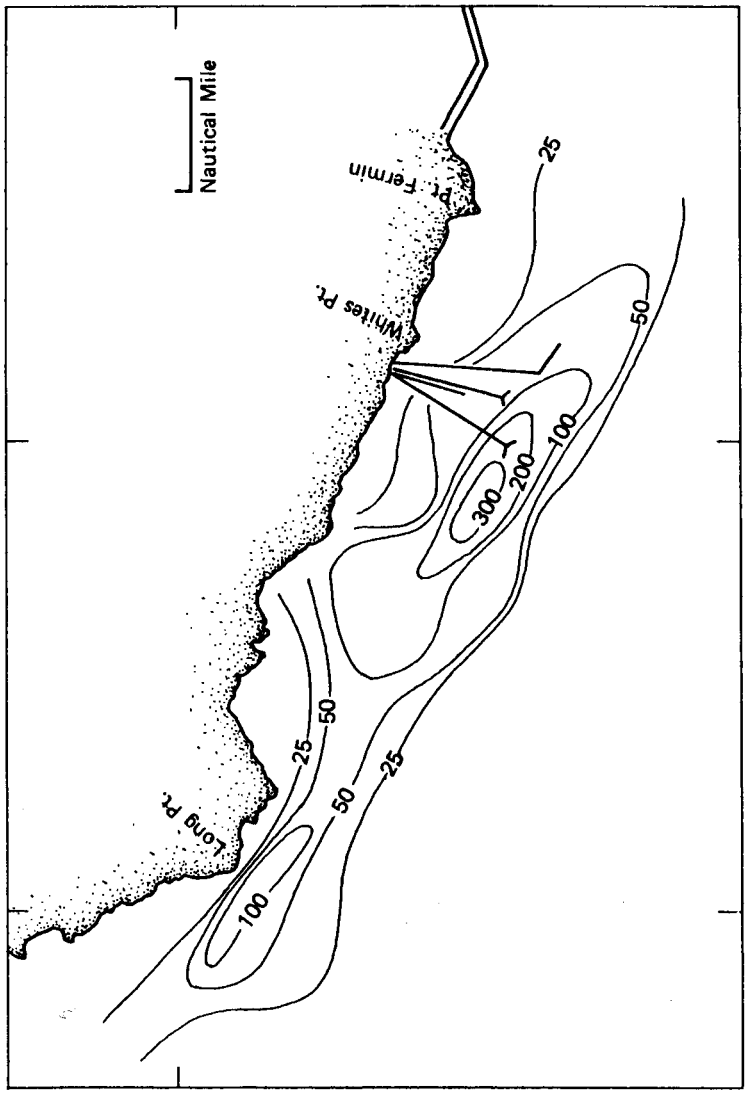

FIGURE 6 Distribution of the total DDT concentrations in surface sediments at the Palos Verdes Shelf near Los Angeles, California. Units are in 10^{-9} g/g (ppb) (From D. J. McDermott, T. I. Heesen and D. R. Young, 1974. DDT in bottom sediments around five Southern California outfall systesm. SCCWRP, TM 217).

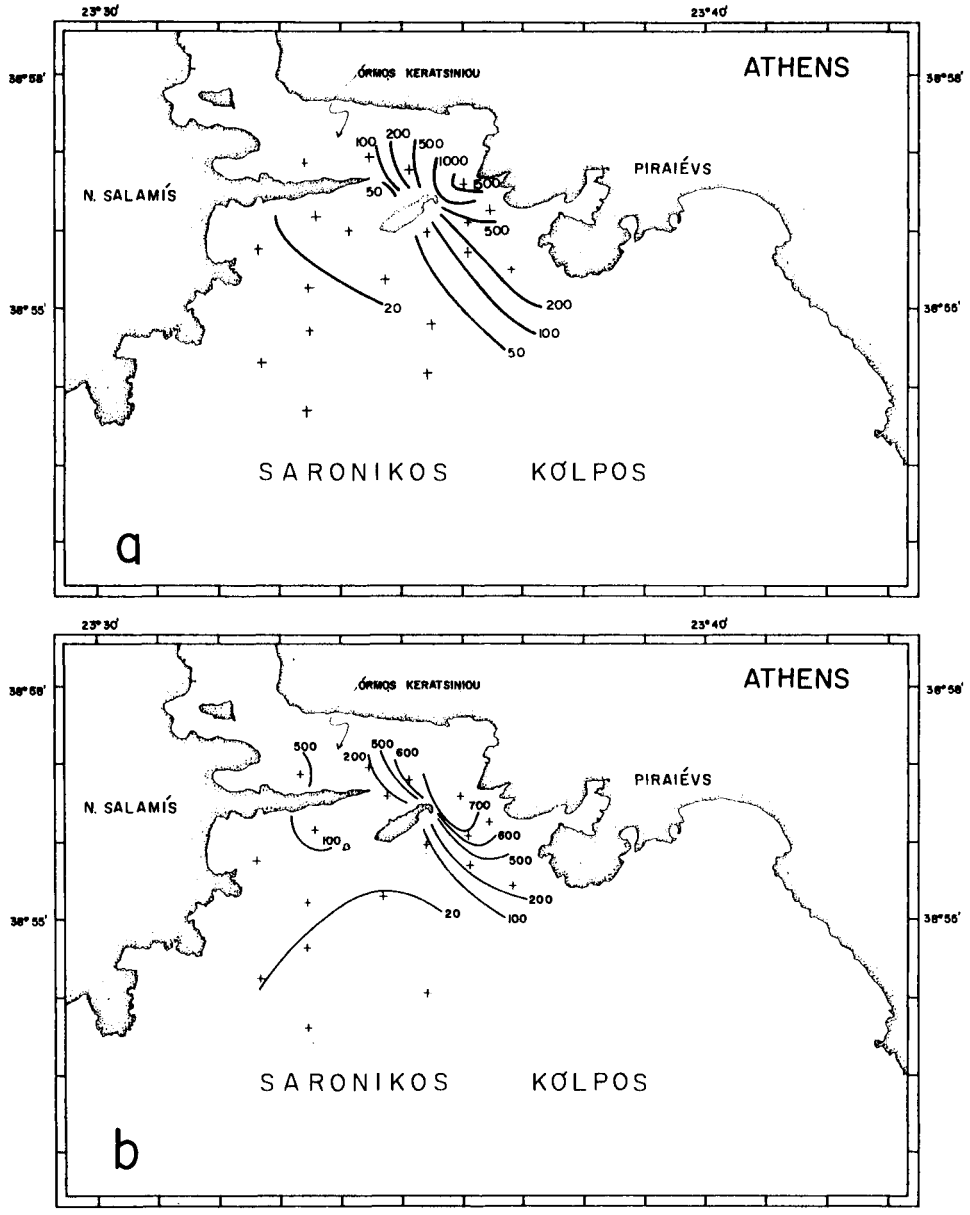

FIGURE 7 Distribution of chlorinated hydrocarbons in surface sediments of the Kerantsini Bay and Upper Saronikos Gulf in Southern Greece. Concentrations are expressed in 10^{-9} g/g (ppb); a, total DDT; b, total PCBs (from R. N. Dexter and S. P. Pavlou, chlorinated hydrocarbons in sediments from Southern Greece, Marine Pol. Bulletin, 4(12):188-190, 1973).

A Method of Data Analysis on the Distribution of Chemical Elements in the Biosphere

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ABSTRACT

The analytical data on the distribution of chemical elements in the biosphere is promptly increasing in numbers. A method of data analysis which can arrange these data in accordance with a definite principle has been proposed. It was found that there was a linear relationship between logarithm of concentration factor of elements in seaweeds and logarithm of oceanic residence time of elements. Along with some mathematical treatment of the regularity, both characteristics of each algal species and the behavior of each element on the general distribution in the seaweeds could be expressed. It was also found that this regularity between residence time and concentration factor occured in other phyla of marine organisms such as marine phytoplankton and zooplankton. Furthermore, since the ocean is closely connected with each of geochemical spheres, there is a tendency that the value of oceanic residence time has similar relationship with the concentration ratio of many other geochemical abundance to oceanic chemical abundance as well as with concentration factor of marine organisms.

INTRODUCTION

A systematic investigation of chemical elements in various Japanese seaweeds has been performed by the authors (Yamamoto and Ishibashi, 1971). A general regularity between concentration factor of elements in seaweeds and oceanic residence time of elements emerged from the results (Yamamoto, 1972), and has been discussed from the viewpoint of chemotaxonomy (Yamamoto and the co-workers, 1978a, 1978b). In environmental science, the analytical data on the distribution of chemical elements in the biosphere is promptly increasing in numbers. FAO of the United Nations has promoted the program to make an inventory of data on contaminants in aquatic organisms. The method of data analysis which can arrange these data in accordance with a definite principle will be necessary in this field. Biosphere consists of living matters and their environmental substances which comprise parts of hydrosphere, lithosphere and atmosphere. Therefore, it is important to set up an appropriate standard which can use for mutual comparison among the analytical data of various substances in the biosphere. Since the ocean is closely connected with each of geochemical spheres,

it will be expected that oceanic residence time can use as a standard for the general data analysis. The purpose of this paper is to propose a general method of the data analysis which was induced on the base of the results of Japanese seaweeds.

EXPERIMENTAL

Material and Analytical Procedure

Samples of seaweeds, marine phytoplankton, marine zooplankton and limnetic weeds were washed throughly with distilled water and dried. Before analysis, the samples were dried to constant weight at 105°C, and ashed in a muffle furnace at 500°C. The ashed samples were analysed. A method was selected for each element from spectrophotometry, gravimetry and polarography (Yamamoto and the co-workers, 1978b).

Data and Definitions

Data on seaweeds (Yamamoto and the co-workers, 1978b), marine plankton (Fujita and the co-workers, 1969; Fujita, 1971, 1972; Yamamoto and the co-workers, 1973) and limnetic weeds (Yamamoto and Shimada, 1970) were used from the results obtained in the author's laboratory, while data on human body (Morgan, 1970), rain water (Sugawara, 1964), hot spring (Kitano, 1964), Clarke value (Taylor, 1964) and chondrite (Vinogradov, 1962) were cited from the literatures. The definitions of oceanic residence time x and concentration factor y were adopted as follows.

$x = A / dA/dt$

A : the total amount of element in suspension or solution in the ocean

dA/dt : the amount introduced or precipitated per unit time

y = ppm in wet marine organisms / ppm in sea water of the element concerned (assuming that marine organisms contain 75% water)

In the cases of limnetic weeds, human body, hot spring, rain water, Clarke value and chondrite, the concentration ratio of each substance to sea water was substituted for concentration factor. In the following calculations, the values of residence time and chemical abundance of sea water cited from the recent investigation (Goldberg and the co-workers, 1971).

Calculation

A result on seaweeds was illustrated in Fig. 1. It shows that there is a linear relationship between logarithm concentration factor y and logarithm oceanic residence time x. Consequently, the following formulas can be assumed.

$$y = a x^b \qquad (1)$$

$$\log y = \log a + b \log x \qquad (2)$$

In Fig. 1, the values of a and b were calculated by the method of the least squares from the formula (1), and the straight line was drawn by the linear equation (2). The log a was the line's intercept with the ordinate and b was its slope. Correlation coefficient r between log x and log y was also calculated from the original data. When the values of a and b could be known, theoretical value of concentration factor y_o for each element was calculated from the assuming formula (1) corresponding to a definite x value.

RESULTS AND DISCUSSION

The concept of oceanic residence time was introduced by Barth (1952) and was confirmed by Goldberg and Arrhenius (1958). It assumed an ocean as a steady state in which the amount of a given element introduced per unit time was compensated by an equal amount deposited in the sediments. Accordingly, the value of dA/dt in the definition had been calculated from the averaged composition of either river water or marine sediments. In the present phase of marine chemistry, the concept of oceanic residence time has been widely accepted as an indication of geochemical reactivity which elements exhibit in sea water. On the other hand, the importance of the concentration of trace elements to marine organisms from sea water has been appreciated for a long time. From the definition, it can be said that the concentration factor reflects biochemical reactivity. This means that elemental uptake into algae from the surrounding sea water is concerned with the relative reactivity of the elements in sea water. According to the recent papers (Bewer and Yeats, 1977; Fowler, 1977), it is also becoming to be acceptable that biological activity of marine organisms has important effects on the values of oceanic residence time. Therefore, it is reasonably presumed that there may be any connection between oceanic residence time and concentration factor of marine organisms.

Figure 1 shows the illustration on individual species of seaweeds (Eisenia bicyclis). Each of the elements takes inherent positions relative to the straight line, but there is an excellent linear relationship between log x and log y on the whole. By the way of selection of elements for this calculation, some variation appeared in the values of log a, b and r even on the same species. However, the variation was not so great, if the data was chosen properly. Therefore, these numerical values could use as an indication of the charateristic of each species on the distribution of the chemical elements. In order to give a representative indication for each species, the kinds of elements should be selected throughout a wide range of residence time x. The characteristics of these numerical values on various species of seaweeds were discussed in the proceeding paper (Yamamoto and the co-workers, 1978b).

Figure 2 shows the linear relationship between logarithm concentration factor y of marine zooplankton and logarithm oceanic residence time x. Since zooplankton is chiefly fed with small algae, the existence of the relationship in the phylum is presumable. Table 1 shows the numerical values of log a, b and r on each order of marine zooplankton. The absolute values of r were more than 0.87 through the nine orders, and the values of log a and b were characteristic on each classification. Table 2 shows a list of y/y_o for each element on the nine orders of marine zooplankton. The values of y/y_o indicate the actual concentration factor of each element with respect to the theoretical value. Some elements such as phosphorus and zinc had high values, while other elements such as iron and aluminum had low values. Moreover, the values for each element were limited within a small range among the phylum. Therefore, the values of

y/y_o could be an indication of a characteristic of each element on the distribution in the planktonic body through various species of marine zooplankton.

Figure 3 shows the relation between concentration factor y of marine phytoplankton and oceanic residence time x. There is an excellent correlationship between log x and log y in this phylum of marine organisms. Since marine phytoplankton has high contents of trace elements such as iron and zinc, the values of log a and -b were relatively higher than those of seaweeds. Figure 4 shows the relation between concentration ratio y of limnetic weeds to sea water and oceanic residence time x. Though the aquatic samples were not actually living in the sea, the concentration ratio of limnetic weeds to sea water still had a good correlationship with oceanic residence time. The existence of the correlationship might have the relation with the fact that the limnetic weeds had inherently evolved out of the marine plant.

In the following illustrations, it was found that such a linear relationship between log y and log x exists in a wide range of biosphere. Table 3 shows a list of log a, b and r values on various living matters and environmental substances. Though absolute value of r of human body was relatively lower than those of other substances, there was still an appreciable linear relationship between log x and log y. It might suggest that the origin of our life would have connection with the ocean. Namely, the coexistence of those general relationship in living matters might be based on the fact that there is a mutual resemblance of elemental composition among various living matters.

In the definition of oceanic residence time, values of dA/dt had been estimated from the data on the average composition of river water and the total amount of river water delivered into the sea annually (Barth, 1952). Therefore, assuming the mutual resemblance of elemental composition between river water and other substances of hydrosphere, the high absolute value of r on rain water and hot spring are presumable. On the other hand, values of dA/dt in the definition of oceanic residence time had been alternatively estimated from the data on the median rate of marine sedimentation and average composition of marine sediments. Goldberg and Arrhenius (1958) assumed the composition of marine sediments to be the same as crustal rock in their calculation. Therefore, assuming the mutual resemblance of elemental composition between crustal rocks and other substances of lithosphere, the high absolute value of r on Clarke value and chondrite are presumable. Although there is such a common linear relationship in geochemical substances tested, the values of log a and b were characteristic for each living matter and environmental substance respectively. Table 4 shows a list of y/y_o value for each element on the living matters and environmental substances. It can be seen that the values of some elements show a remarkable difference among the substances.

Both oceanic residence time and concentration factor are essentially due to experimental results. Therefore, the results on proposed mathematical treatments should improve with advance of experiments. For a general use, reliable values of oceanic residence time on selected element should be adopted as a standard in this method.

TABLE 1. List of log a, b and r values on Marine Zooplankton

Order	Number of samples	Number of elements	log a	b	r
Trachylina	2	10	7.1	-1.00	-0.88
Sagittidea	7	10	7.4	-1.06	-0.89
Cladocera	2	9	8.1	-1.14	-0.89
Copepoda	32	11	7.5	-1.04	-0.93
Amphipoda	7	11	7.1	-0.98	-0.91
Decapoda	1	8	8.6	-1.13	-0.93
Mysidacea	1	9	7.6	-1.04	-0.88
Euphausiacea	7	10	7.0	-0.96	-0.87
Thaliacea	3	11	6.9	-0.84	-0.90

TABLE 2. List of y/y_o values on Marine Zooplankton

Order	Al	Fe	Mn	Si	Zn	P	Ca	K	Mg	B	Na
Trachylina	-	0.2	0.8	0.2	10.4	144	0.05	1.5	0.6	1.3	3.4
Sagittidea	-	0.2	0.9	0.2	6.1	201	0.07	1.6	0.7	0.9	0.3
Cladocera	-	0.4	0.3	0.5	3.9	248	0.06	1.3	0.3	-	0.9
Copepoda	0.4	0.4	1.4	0.3	5.3	90	0.1	1.2	0.4	1.9	0.3
Amphipoda	0.2	0.3	2.0	0.4	7.1	164	0.2	1.0	0.4	1.2	0.2
Decapoda	-	0.2	1.5	-	3.5	41	0.08	1.3	0.3	-	1.0
Mysidacea	-	0.3	0.6	0.09	7.8	158	0.3	1.4	0.6	-	0.2
Euphausiacea	-	0.2	0.7	0.2	7.5	142	0.1	0.7	0.2	7.2	0.2
Thaliacea	0.2	0.4	3.3	0.4	4.2	110	0.1	1.4	1.0	0.6	0.2

TABLE 3. List of log a, b and r on Biosphere

	Number of samples	Number of elements	log a	b	r
Seaweeds	221	20	7.1	-0.91	-0.94
Marine Phytoplankton	27	11	8.9	-1.19	-0.95
Marine Zooplankton	65	11	7.4	-1.01	-0.93
Limnetic Weeds	19	11	8.1	-1.15	-0.97
Human Body		17	5.9	-0.82	-0.79
Rain Water		13	4.3	-1.09	-0.98
Hot Spring		10	6.6	-1.08	-0.96
Clarke Value		20	10.9	-1.38	-0.96
Chondrite		20	11.5	-1.51	-0.89

TABLE 4. List of y/y_o values on Biosphere

	Al	Fe	Mn	Ga	Ti	Si	Cu	Zn	Cr	V	Ni	Co	P	Mo	Ca	Sr	K	Mg	B	Na
Seaweeds	0.8	0.4	4.2	0.4	4.2	0.3	0.8	2.6	0.4	1.0	0.3	2.3	14.9	0.7	0.3	3.7	1.2	0.5	1.8	0.2
Marine Phytoplankton	0.1	0.7	2.1			2.6		2.7					24.0		0.08		8.7	0.4	1.3	0.1
Marine Zooplankton	0.3	0.4	1.8			0.3		5.6					111		0.1		1.1	0.4	1.6	0.3
Limnetic Weeds	0.8	0.7				0.4		3.2			0.5	9.6		0.4	3.0	0.3		0.5	1.4	
Human Body	0.05	1.9	0.2			0.3	1.4	13.6	0.7		0.2	1.1	2800	0.8	3.5	0.2	2.6	0.2	0.2	0.4
Rain Water	0.7	1.1				0.5	0.6	0.9		6.8			3.7	0.6	0.4	1.0	0.8	0.6		1.4
Hot Spring	0.3	7.0	5.2	0.4			2.6	0.1							0.3	1.3		0.4		4.2
Clarke Value	0.6	0.3	1.8	1.9	31.2	0.8	0.2	0.07	2.0	4.5	0.9	10.9	2.4	0.2	0.2	0.7	1.7	1.2	0.3	1.6
Chondrite	0.04	0.7	3.3	0.3	2.4	0.5	0.3	0.05	46.3	2.6	173	427	1.4	0.1	0.1	0.03	0.1	15.6	0.1	1.3

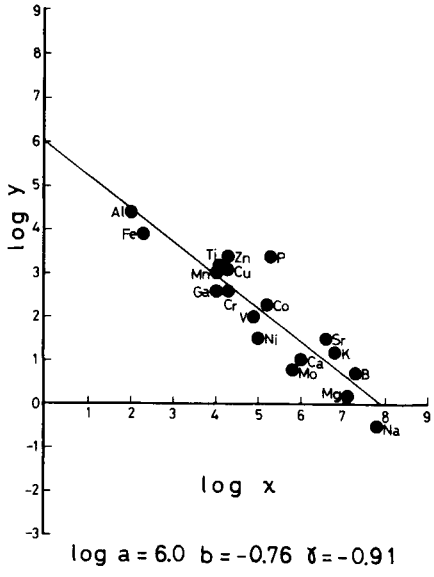

Fig. 1. Eisenia bicyclis
(mean on 9 samples)

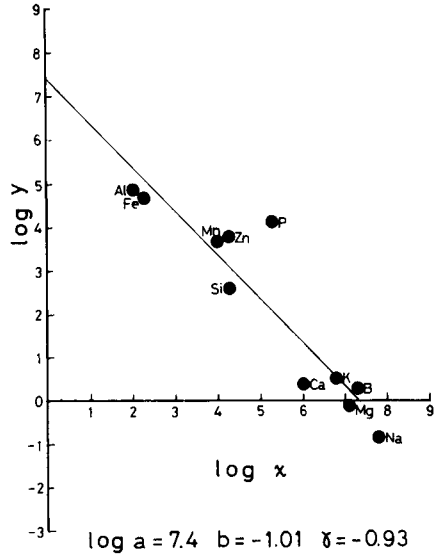

Fig. 2. Marine zooplankton
(mean on 65 samples
of 35 species)

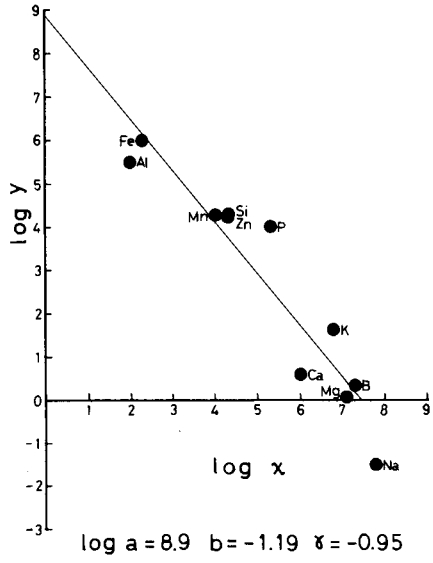

Fig. 3. Marine phytoplankton
(mean on 27 samples)

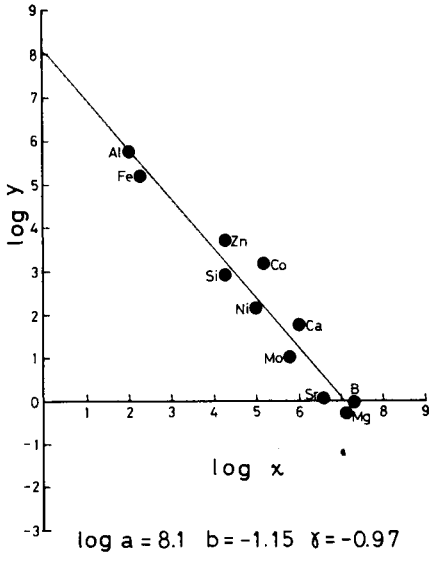

Fig. 4. Limnetic weeds
(mean on 19 samples
of 10 species)

CONCLUSION

A general method of data analysis on the distribution of chemical elements in the biosphere was introduced. The values of log a, b and r could use as an indication of the character of each biogeochemical substance on the distribution of chemical elements, while the value of y/y_o could be an indication of a characteristic of each element on the general distribution through living matters or environmental substances.

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Chemical Changes in the Sediments of Loch Eil Arising from the Input of Cellulose Fibre

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ABSTRACT

Studies of the effect of a particulate cellulose effluent from a pulp and paper mill on the sediments of Loch Eil have shown that redox measurements taken at a series of stations can be used to map those areas of sediment exhibiting high reducing activity. The extent and variations in these areas is closely related to the input levels of waste cellulose fibre and provides a useful indicator of the 'health' of the sediment. Some of the changes in sediment chemistry resulting from increased input of fibre give rise to an increase in sediment sulphide levels. Changes also occur in the nature and amount of the low molecular weight acids formed in the sediment during cellulose degradation. In the area of highest deposition both acetate and succinate are found, but the highest levels of acetate are present in areas of intermediate deposition. The measurements made on the above parameters are discussed in relation to their use in assessing the short term changes in sediments subject to variable inputs of organic pollutants.

INTRODUCTION

The possible environmental effects of the effluent from a pulp and paper mill on the marine ecosystem of Loch Eil (Fig. 1) led to the establishment of a long term biological study of the sediments of the loch which was initiated prior to the building of the pulp mill (Pearson,1970,1971,1972,1975).

Studies on the benthic populations of Loch Eil have shown significant variations in the biomass and species composition in response to changes in organic input over the last ten years (Pearson,1971,1975). However the response time of such population changes to environmental perturbations is relatively slow in all but catastrophic situations and thus repetitive population analyses do not provide a rapid means of assessing the health of the sediments.

A number of chemical techniques were therefore used in an attempt to assess short term changes in the sediment which could be related to subsequent biological changes. Long term analysis of cellulose, the major component of pulp fibre, showed no significant short term changes in the monthly levels

though there were significant differences between the different sampling stations. Since it can be shown that there is very little net accumulation of cellulose in the sediments (Stanley et al.1978),any method for assessing short term changes must rely on the ability to detect change occurring as a result of cellulose breakdown. Similar considerations apply to the use of total carbon and carbon to nitrogen ratios. It has also been shown in experimental tanks loaded daily with different amounts of pulp fibre that after a period of six months there is no difference in cellulose levels between the loaded and the control tanks. In other words, analysis for cellulose, the major organic input, gives no indication of its accumulative effect on the sediments.

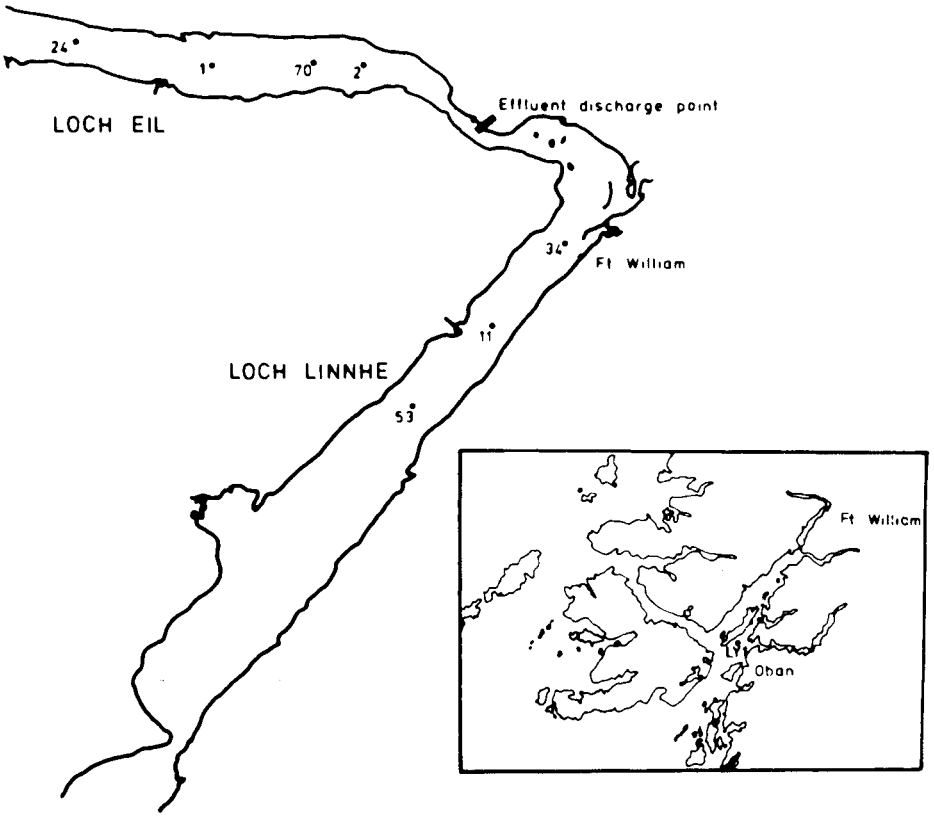

Fig. 1. Map of sampling stations in Loch Eil and surrounding area

The microbial degradation of cellulose is basically an oxidative process in which electrons are removed and then transferred to electron sinks. Oxygen is the most important sink but when this is used up other sinks are used. In marine sediments sulphate is a key sink since it is used by the sulphate

reducing bacteria. During cellulose degradation the available sinks are used up and unless they are replaced, or the input rate is lowered, reduced degradation products of cellulose will accumulate in the sediment and this will be reflected by a fall in the redox potential (Whitfield,1969,1971). Extensive measurements made over a series of stations in Loch Eil showed a relationship between the input of pulp fibre and the area of the loch characterised by highly reduced sediments. Similar experiments conducted in experimental tanks loaded with different amounts of pulp fibre showed that the higher the loading of fibre the more rapidly the sediment became reduced but that on cessation of loading the sediments became progressively less reduced, reaching their original unloaded values in about six months (Stanley et al.1978) Though redox measurements, as used in these studies, do not provide data which is strictly interpretable in chemical terms (Whitfield,1969,1971) they do provide a rapid and semi-quantitative assessment of the short term response of the sediments to organic pollution.

Various other techniques have also been used to complement the redox measurements made on Loch Eil sediments. Analysis of sulphide levels show significant variations between the different stations and short term changes were also shown in sulphide levels,but it was not possible to relate this directly to the input of pulp fibre (Stanley et al.1978). A study was also undertaken of some of the low molecular weight acids formed during cellulose degradation which are possible indicators of processes taking place in the sediment; marked differences were shown both in the amounts and distribution of the various acids depending on the level of cellulose input (Miller et al. 1979). This paper describes results obtained from the various chemical techniques used in studying the Loch Eil sediments and discusses their applicability to assessing short term changes induced by organic pollution.

METHODS

Sediment samples for chemical analysis were taken using a Craib corer (Craib, 1965) from stations E70 and E24 in Loch Eil and from the control station LY1 in the Lynn of Lorne (Fig. 1). A description of these stations can be found elsewhere (Miller et al.1979). Core samples for redox measurements were taken over a series of 24 stations in Loch Eil.

Redox measurements were made by inserting specially constructed combination glass electrodes to different levels in the core and reading the mV on a digital pH meter. The electrodes contained a Ag/AgCl reference element and were manufactured by Russell pH Ltd., Auchtermuchty,Scotland (Type No: CMF 2/250/Mod R2). Th electrodes were standardised against a ferrocyanide-ferricyanide buffer (Zobell,1946) and the potential corrected relative to a standard hydrogen electrode. Detailed information on the use of these electrodes can be found elsewhere (Pearson and Stanley,1979).

Soluble sulphide was determined by a modification of the method described in Applications Bulletin No:12(Orion Inc.,11 Blackstone St.,Cambridge, Mass. 02139, USA). 10 or 20ml samples of sediment were taken from extruded Craib core sections using a cut down plastic syringe and immediately mixed with an equal volume of sulphide anti-oxidant buffer (SAOB. Orion Bull. No:12). An Orion sulphide specific electrode and a double junction reference electrode (Model No:94-16 and 90-02 respectively) were immersed in the sediment slurry and magnetically stirred. The electrode potential was read on a digital voltmeter after the potential had stabilised. The electrodes were

calibrated in serially diluted standards prepared from 0.1M Na_2S in sulphide anti-oxidant buffer. The Na_2S was standardised by titration against 0.1M $Pb(NO_3)_2$. A calibration graph was prepared by plotting electrode potential against molarity on semi-log paper and the concentration of soluble sulphide in the sediment samples determined from this after correcting for dilution by the buffer.

Analysis of low molecular weight acids was done as desribed elsewhere after extraction of the pore water by squeezing (Robbins and Gustinis,1976) or by the insertion of dialysis bags into the experimental tanks and subsequent analysis of the contents (Miller et al.1979).

Samples of sediment for carbon, nitrogen and cellulose analysis were obtained by extruding core samples out of a core tube and cutting into 3cm sections. The sections were then dried at 70°C for 48hr and ground to a fine powder in a high speed laboratory mill. Cellulose was determined by a modification of the method of Updegraff (1969) and is described in detail elsewhere (Stanley and Pearson,1979). Analysis of total organic carbon and nitrogen was performed on a Perkin-Elmer Elemental Analyser (Model No:240; Perkin Elmer Inc.,Norwalk, Conecticut,USA) after prior treatment of the sample with hydrochloric acid to remove inorganic carbon.

RESULTS

An average of monthly readings of carbon, nitrogen and cellulose in the surface sediments of Loch Eil (Table 1) shows that the Loch Eil sediments contain much higher levels of carbon than the control station LY1 but that the C:N ratio increases as the amount of carbon input to the sediment is increased with the highest C:N ratio being found in the deep basin of Loch Eil where the fibre deposition rate is greatest.

TABLE 1. Carbon, nitrogen and cellulose in Loch Eil Sediments

Station	Carbon (%)	Nitrogen (%)	C:N	Cellulose (mg/g/dry wt.)
LY-1	2.90	0.37	7.84	0.62
E-24	5.27	0.46	11.46	3.29
E-70	6.18	0.46	13.43	4.33

Results are the average of monthly readings over several years from the top 0-3cm layer of core samples.

Measurements of redox made at a series of stations at 200m intervals throughout Loch Eil are presented as a map showing the area of highly reduced sediment(less than -100mV) and this area can be shown to be highly mobile and related to the suspended solids input to the loch system (Fig. 2). This relationship is seen more clearly when suspended solids output is plotted against area of the loch sediment having a potential of less than -50mV(Fig.3).

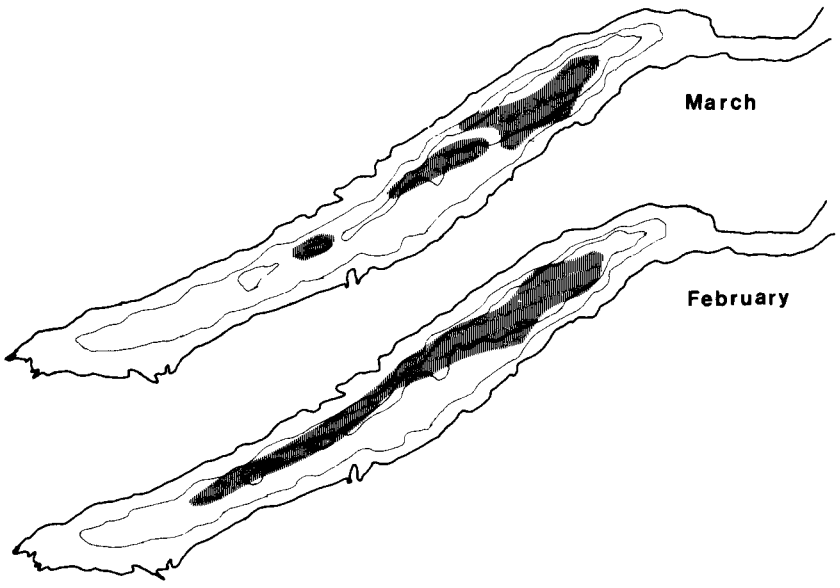

Fig. 2. Redox maps of Loch Eil showing the extent of highly reduced conditions in February 1977 following monthly inputs of 19 tonnes/day in January and 15 tonnes/day in February compared with March 1977 following monthly inputs of 15 tonnes/day in February and 10 tonnes/day in March.

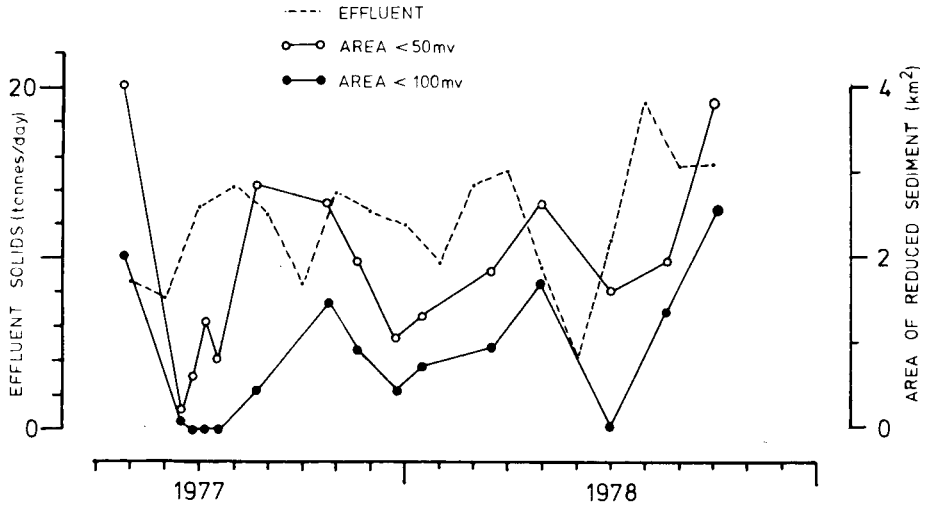

Fig. 3. Relationship between areas of reduced sediment and average monthly inputs of suspended solids in 1977-78

Soluble sulphide was also measured in sediments at E70,E24 and LY1 during 1975-1976 (Fig. 4) and compared with suspended solids input. Sulphide was not detected in more than trace amounts at LY1. Consistently higher values for sulphide were found in the deep basin of Loch Eil(E70) which had the highest fibre input, compared with the station at the head of the loch(E24). However the relationship between sulphide levels and suspended solids input does not follow the same pattern in both areas of the loch,and this may reflect differential deposition of effluent material in response to local hydrographic conditions

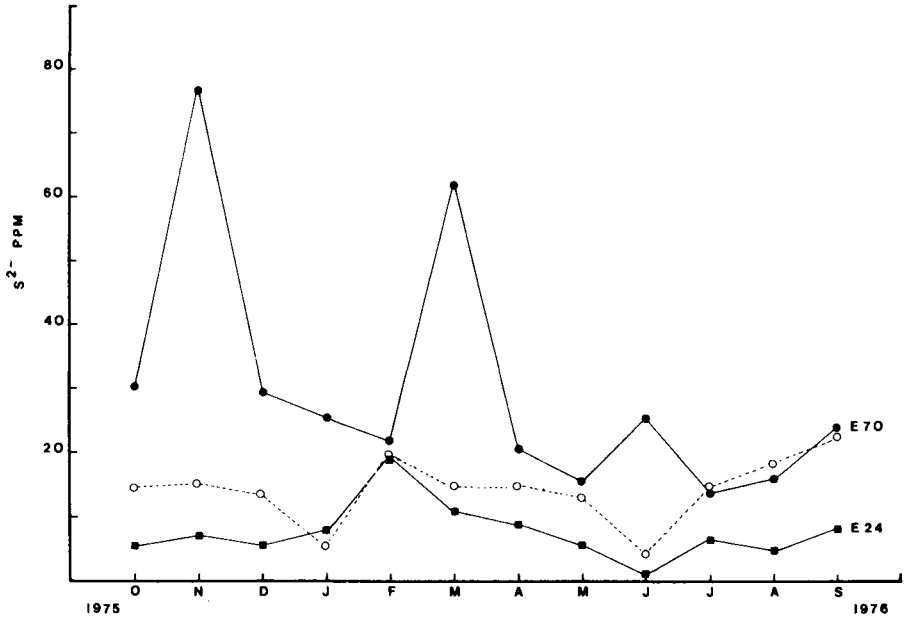

Fig. 4. Soluble sulphide levels at stations E24 and E70 in Loch Eil compared with average monthly suspended solids input.(Suspended solids in tonnes/day on same scale as S^{2-}). Dotted line is suspended solids.

Analysis of low molecular weight acids extracted from pore water show significant differences between the sampling stations (Table 2). In the area of highest deposition both acetate and succinate are found but the highest amounts of acetate are found at E24 where the deposition rate is lower. Lower amounts of acetate are found in the control station LY1. Succinate was not found in samples from E24 and LY1.

A study was then made of acid levels in experimental tanks loaded with different amounts of pulp fibre (Fig. 5 and Table 2). These showed that the acetate level increased with increasing input of pulp fibre to reach a peak at input levels of 1.50g/m^2/day of fibre. At loading levels above this acetate decreased and significant amounts of succinate appeared,thus simulating the conditions prevailing in the deep basin of Loch Eil at E70.

DISCUSSION

A number of techniques have been used during this study in an attempt to assess the health of the sediments of Loch Eil and their response to different

levels of organic input.

Direct chemical measurement of carbon, nitrogen, C:N ratio and cellulose give no indication of short term sediment response,and as has been shown from experimental tank studies cellulose levels do not reflect the degree of pulp loading (Stanley et al.1978). Nevertheless these measurements do provide useful information in describing the general organic status of the sediments and the C:N ratio gives some information as to the nature of material reaching the sediment surface. High C:N ratios probably reflect the input of predominately cellulose fibre which has a C:N ratio of about 33:1 (Vance,1978) and the low C:N ratio reflects the predominately plant inputs at LY1.

TABLE 2. Dissolved organic acids in Loch Eil sediments and in experimental tanks

Station	Acid concentration in µg/g dry wt. sediment					
	Acetate	Propionate	Butyrate	Valerate	Lactate	Succinate
LY-1	322	nd	1.5	tr	27.2	nd
E-24	1836	30.6	24.0	160.0	14.4	0.1
E-70	780	59.0	30.7	24.0	21.4	42.2
Cellulose added (g/m^2/day)	Acid concentration in experimental tanks in µg/ml of dialysate					
Control	148	nd	3.0	nd	nd	nd
0.50	284	tr	10.0	29.0	tr	nd
1.50	517	tr	0.2	nd	-	-
2.25	244	3.7	8.0	tr	3.8	10.0

Data adapted from Miller et al. 1979)

Redox measurements on the other hand, appear to respond relatively rapidly to changes in the organic input to the sediment and reflect the increased oxygen demand of the sediment as the input levels are increased. However, redox measurements as used in this work, provide only a semi-quantitative measurement and cannot be interpretated in strictly chemical terms since they reflect a whole series of complex oxidation-reduction reactions taking place in the sediment(Whitfield,1969,1971).

The measurement of sulphide as an environmental indicator is based on the fact that sulphide is formed from the microbial reduction of sulphate and for this to take place the substrates for sulphate reduction must be present. Such substrates are likely to be formed during cellulose degradation and the greater the cellulose degradation the greater the sulphate reduction. Thus

the highest levels of sulphide are found in the deep basin of Loch Eil where cellulose levels are highest and conversely at the control station LY1 where the natural carbon inputs are low and there is no additional input of organic effluent, no sulphide is detectable in anything but trace amounts. However the measurements of sulphide as used in this study can only give a semi-quantitative picture since the method as used here is subject to error. It has recently been shown that small differences in pH(less than 0.1)between the sample and standards can produce large errors (Mosey and Jago,1977). An alternative method suggested by these workers uses 10M KOH to overcome the problem of sensitivity to small fluctuations in pH of the sample but,apart from the difficulty of working with 10M KOH,this will also cause the solution of the insoluble metal sulphides. Nevertheless, significant variation in sulphide levels can be seen throughout the year at the stations sampled which may be directly or indirectly related to the organic input from the mill effluent. However, it has not been possible to relate sulphide measurements consistently to short term changes in organic deposition rates.

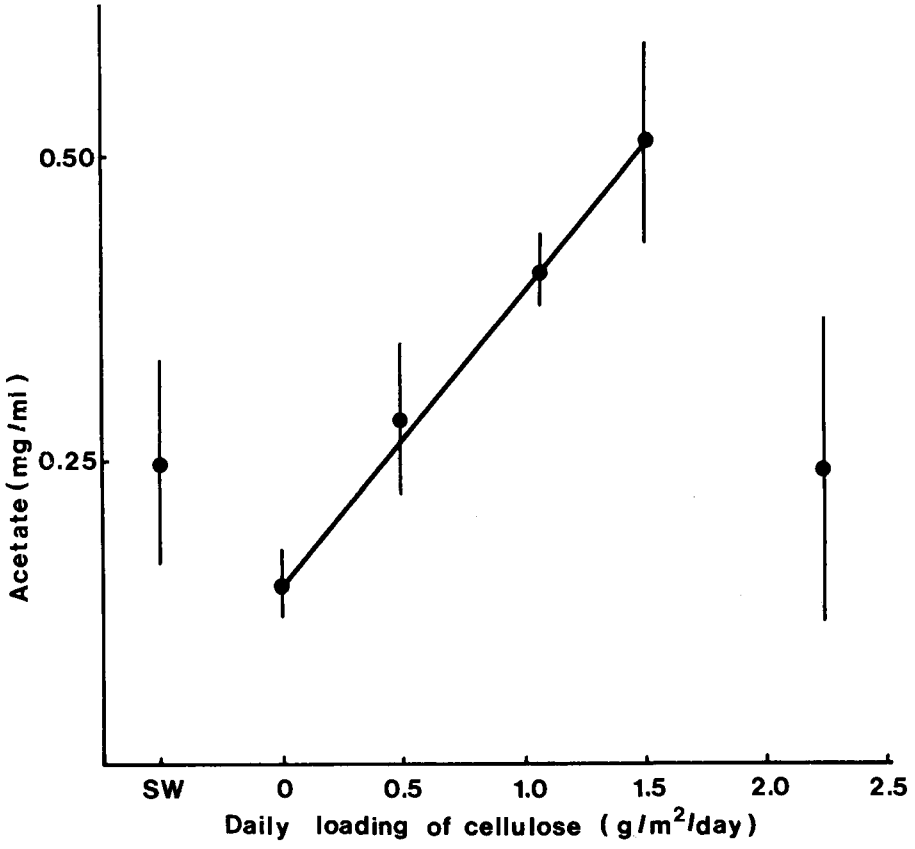

Fig. 5. Change in acetate levels in dialysate bags placed in experimental tanks loaded daily with different amounts of pulp fibre. SW = seawater input.

As a result of previous work in this laboratory (Vance,1978) the low molecular weight organic acids formed from the the degradation of cellulose, some of

which are likely to be substrates for, or products from, sulphate reduction, were analysed in sediments from Loch Eil. These acids may provide important indicators of the processes taking place in sediments (Cappenberg,1974). The high concentrations of acetate found at all stations and the fact that, in experimental tanks, the amount of acetate formed appears to be related to load level, suggest that this compound might prove a useful indicator of sediment condition. The appearance of succinate in the highest loaded experimental tank, and in station E70 in Loch Eil, suggest that succinate might also be an important chemical indicator of excessive organic loading for reasons described elsewhere (Miller et al.1979). Though measurements of low molecular weight organic acids appear to hold considerable promise in assessing the microbial processes taking place in the sediments of Loch Eil they are technically difficult analyses to do and are not as yet suitable for routine field measurements.

Despite the criticism of it on chemical grounds (Whitfield,1969,1971), redox measurement as used in this study provides the most rapid and convenient way of assessing short term changes in sedimentary conditions in response to changing inputs of biodegradable organic pollutants.

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Organic Carbon in a Scottish Sea Loch

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ABSTRACT

The relatively pollution free Scottish sea lochs are excellent water systems in which to study the dispersal of organic matter. We have tried, through measurement of total organic carbon (TOC), particulate carbon (POC) and other parameters in Loch Creran, to develop a model for the flux of organic carbon which may be applied to a number of sea lochs. TOC was measured using ultra-violet combustion of water samples in an automated system. The distribution of the TOC in the loch and its rivers is described and a budget for TOC based on our measurements and others' is presented. The mathematical model based on the distribution of TOC is also described and a comparison of this model with the loch itself is discussed.

KEYWORDS

Organic carbon, Sea loch, Carbon budget, Modelling.

INTRODUCTION

The fjordic sealochs of the West Coast of Scotland are increasingly becoming places of scientific, commercial and recreational interest. They are relatively undisturbed areas with features which make them ideal for the study of marine plants and animals and the physical and chemical processes which affect them. They are enclosed bodies of sea water which offer a good environment for the farming of many types of fish and their generally large supplies of fresh water make them attractive to various industrial developments. It is therefore important that we understand the water systems themselves and the ways they are affected by human usage.

We describe here measurements of various quantities in Loch Creran and its associated river system. The loch is on the west coast of Scotland near Oban in Argyll. The main basin of the loch is separated from the sea and from the smaller upper basin by shallow sills (Fig. 1).

Our aim is to construct a useful model of the flux of organic carbon through the Loch so that future perturbations to its input or to the factors which affect its distribution may be assessed. Besides the results of our own measurements we have drawn on the work done in Loch Creran on the primary

production of phytoplankton (Tett and Wallis, 1978) and of the sea weed (Johnston, Jones and Hunt, 1977). The estimation of the levels of terrestial and industrial/domestic input and distribution of organic carbon in the loch are the remaining pieces of the puzzle.

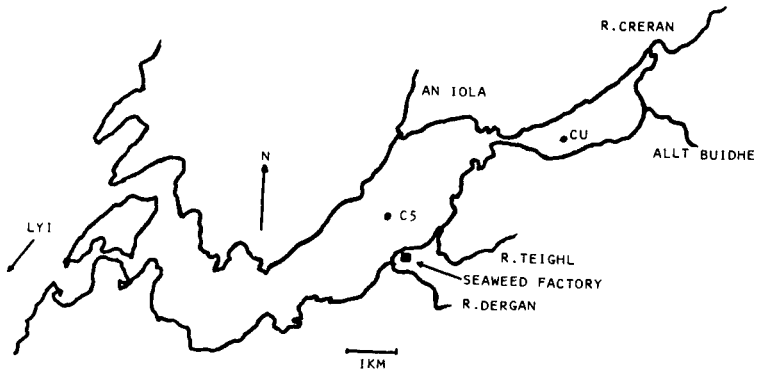

Fig. 1. Map of Loch Creran, about 15 km north of Oban. Lat. $56^{o}32'N$ Lon. $5^{o}23'W$, showing principle rivers, location of sampling stations and of seaweed processing plant.

EXPERIMENTAL

Fieldwork

Sampling was carried out fortnightly in Loch Creran using the S.M.B.A. research vessel 'Seol Mara'. Trips were planned to coincide as nearly as possible with neap tides. Water samples were collected from three depths at each of three stations Fig. 1, using standard N.I.O. water bottles, and transferred directly into vacuum flasks. Temperature and salinity were measured with an E.I.L. temperature-salinity probe and deck unit whose readings were calibrated using laboratory conductivity measurements of water samples from each station and a calibrated thermometer for temperature (Tett and Grantham, 1978).

Total Organic Carbon Analysis

Prior to field trips all glassware was cleaned with permanganic acid to remove traces of organic matter. Aliquots of 25 ml of loch and river water were acidified to below pH 3 with concentrated HCl, 'Aristar' grade (B.D.H. Ltd.) and stored in glass stoppered bottles at $-18^{o}C$. Analyses were carried out on thawed samples using the automated organic carbon analyser of Collins and Williams (1977). The samples were purged of inorganic carbon with a stream of oxygen and then pumped through a narrow bore quartz coil which was continuously irradiated by a 1000 W medium pressure mercury arc lamp. The organic carbon was oxidised to CO_2 which was then stripped from the water by oxygen gas and

measured in a non-dispersive infra-red gas analyser. Water samples were not filtered before analysis. A comparison of particulate carbon determined by difference between filtered and unfiltered samples of the same water and those determined by combustion of filters showed no systematic error was introduced by not filtering water samples.

Particulate Carbon Analysis

Water samples of 500 ml were filtered at reduced pressure (700 mm Hg) through 4.25 cm GF/C glass filters (precombusted at 450°C for 4 hours). The filters were stored in clean glass vials at -18°C until they could be freeze dried for 48 hours. The dried samples were then stored in an evacuated desiccator. The filters were analysed for carbon in a Perkin-Elmer 240 elemental analyser with combustion oven set at 750°C. The procedure was calibrated with weighed acetanilide standards and unused precombusted filters were analysed for a procedural blank.

RESULTS

River Input

The continuous record of water depth in the river Creran, the largest river in the catchment area of the loch, was converted to daily mean flow by the Clyde River Purification Board. The fortnightly measurements of depth in three other rivers could not be converted directly into flow. We assumed instead that their mean daily flow would be equal to the flow of the Creran multiplied by the ratio of their catchment areas to that of the Creran. The flow of a fourth river, An Iola, was estimated similarly from the area of its catchment.

Total organic carbon (TOC) levels have been measured in four of the rivers since April 1978. Particulate organic carbon (POC) has been measured since August 1977. There was no significant correlation found between either TOC or POC and the flow in the river Creran at the time of sampling. Nor was there a correlation between organic carbon and depth in the other rivers. There was a considerable difference between the TOC in the rivers of the upper basin, Creran and Allt Buidhe, and that of the main basin rivers, Teighl and Dergan. The upper basin rivers showed lower levels of total carbon and less variability over the period of sampling than the lower basin rivers. The mean TOC and POC, weighted for the flow volume of the individual rivers, is shown in Table 1. The standard deviation is included to indicate the amount of variability in the data and not to imply that the data is normally distributed. It should be noted at this point that the standard error of the mean of duplicate determinations of TOC had an average value, taken over 140 samples, of 0.10 g/m^3. Only single determinations of POC were carried out.

TOC and POC in Loch Creran

Water samples were taken at three stations; from 2, 8 and 16 m at station C5 in the main basin; from 2, 8 and 25 m at station CU in the Upper basin; and from 4, 16 and 40 m at station LY1 outside the loch in the Lynn of Lorne. The samples were taken on eleven dates from January to September 1978 at either fortnightly or monthly intervals. The total organic carbon values show in general less variability at the nine station-depths in the loch than those in the river. Differences are observed in the nine station-depth mean TOC values and their distributions over the nine month period. The Wilcoxon two sample

TABLE 1 Summary of TOC and POC in Loch Creran Rivers

Basin	TOC[a]	POC[a]	River Flow[b]
Upper	2.56 (0.77)[c]	0.18 (0.11)	0.55
Main	3.31 (1.49)	0.18 (0.23)	0.51

[a]Measured in g/m^3. [b]Units of 10^6 m^3/day.

[c]Figures in brackets are standard deviations.

test for unpaired samples (Sokal and Rolf, 1969) was applied to combinations of station-depth TOC values. This showed that at each station the two lower sampling depths are not significantly different in their TOC levels but that the surface water at each station is different from the deeper water in TOC. There are in effect two types of water at each station, surface water and deep water. This pair testing also established relationships between the surface water in the main basin and the deep water in the upper basin. These results are summarised in Table 2. The numbers in brackets are the variances. Although the Wilcoxon test employs ranking of the data rather than means and variances, the table does reflect the result since the variability of the TOC data is reasonably well represented by a normal distribution. The POC values are included for comparison. These show that quite different influences are governing its distribution.

TABLE 2 Summary of the TOC and POC in Loch Creran

Location	TOC[a]	POC[a]	Model TOC[a]
Lynn of Lorn			
Surface	1.86(.073)[b]	0.17(.009)	1.67(.0019)
Deep	1.49(.020)	0.10(.004)	1.49(-)
Main Basin			
Surface	1.90(.153)	0.33(.034)	1.87(.0065)
Deep	1.74(.057)	0.26(.033)	1.68(.0022)
Upper Basin			
Surface	2.43(.73)	0.32(.035)	1.95(.0125)
Deep	2.04(.184)	0.21(.017)	1.88(.0075)

[a]Measured in g/m^3. [b]Figures in brackets are variances.

DISCUSSION

Inputs of Organic Carbon

The principle sources of organic carbon in the system are the terrestial carbon from the rivers, the primary production of the phytoplankton and the littoral and sub-littoral macro-algae, and the effluent of the seaweed process-ing plant on the shore of the main basin. Domestic input from the few housing developments around the loch is assumed to be negligible and no account

is taken of the possible effects of resuspension of sediments or of diffusion of dissolved organic matter from the sediment.

From average river flow and TOC concentrations (Table 1) we calculated the terrestrial input to the loch to be 1100 tonnes C per year. This estimate may be in error since it is based on the assumption that TOC is completely independent of the river flows and although we have not been able to find a simple relationship it is true that during certain periods of high rainfall large values of TOC were measured. Our best estimate of the range of possible values of this sum is ± 150 tonnes C.

From the data of Johnston, Jones and Hunt (1977) we can calculate the contribution from the kelp beds in the loch. The sub-littoral population is dominated by Laminaria saccharina. The net production of carbon from this source is 76 gC/m^2 in the kelp beds which are estimated to occupy 25% of the area of the loch. The total annual production figure is therefore 250 tonnes per year. This figure is likely to be a low estimate of the total macro-algal production since it neglects the contribution of the inter-tidal species which are in abundance particularly in the upper basin. The area of the inter-tidal zone is some 30% of the area of the loch in the upper basin and about 10% in the lower basin. Assuming the same production rate for intertidal species we could set a maximum of 400 tonnes C for the total macro-algal production and make an estimate of 300 ± 100 tonnes C.

The work of Wood, Tett and Tyler (unpublished) gives a mean annual production from the phytoplankton population in the loch of 60 $gC/m^2/yr$. This gives a total annual production of 800 tonnes C/yr.

Figures for total effluent carbon and effluent volume were kindly provided by management of the seaweed processing plant which discharges into the main basin. Most of the effluent from the factory is in the form of large particles which create a very localised deposit. We have used as an estimate of this fraction a figure provided by S.O. Stanley of the S.M.B.A. (unpublished), for particulate carbon in the effluent. The net "dissolved" carbon calculated from these figures is for our purposes equivalent to a TOC input because of the likely particle sizes included in the "dissolved" fraction. The total carbon input is 3600 tonnes/yr. The net TOC from this is 800 tonnes/yr. There are very large uncertainties associated with this figure since the annual production figures are subject to economic rather than ecological forces and because the contribution of the localised solid effluent to the mobile carbon of the loch through resuspension, diffusion of metabolic intermediates and other processes is unknown. We have taken an arbitrary figure of 100 tonnes as the possible error only to allow for analytical errors and variations in production.

Fate of the Organic Carbon

There are three possible sinks for the organic carbon; sedimentation, exchange with the sea water in the Lynn of Lorn, and respiration in the water column. Another possible sink is the atmosphere when volatile organics evaporate. We have not attempted to investigate this process although it may be a significant route for the respiration and fermentation of the solid effluent of the seaweed processing plant.

We have calculated the rate of passage of TOC to the sea. The net flow of water from the loch is in fact equal to the river flow. The water that passes

to the sea is the surface water of the main basin. This output should then equal the product of the mean TOC level in the main basin surface water and the river flow. This gives a value of 750 tonnes/yr. In addition to this a second quantity is based on a simple hydrographic model of the loch. The dense surface water of the Lynn of Lorne enters the loch on flood tide and sinks into the bottom of the main basin. On the ebb tide the surface water of the main basin flows into the Lynn of Lorne surface water. The mean TOC of the Lynn surface water is 1.86 g/m^3 and that of the main basin surface water is 1.90 g/m^3. The net loss per tidal cycle is therefore the difference between these two times the tidal volume, 29 x 10^6 m^3/day. This gives a value of 420 tonnes/yr. The sum of these two quantities gives us an estimate of the amount of TOC lost to the sea annually of 1200 tonnes C/yr.

The sedimentation of organic carbon in Loch Creran has been investigated by Ansell (1974). No estimation of the true rate of sedimentation was made in the report because of the evidence of resuspended matter at all depths. There is some justification for taking the total carbon sedimented in the spring and summer months as a first approximation of the true rate throughout the year. This gives an annual rate in the main basin of the loch of 50 $gC/m^2/yr$. This is a result similar to that of Steele and Baird (1972), who measured sedimentation in Loch Ewe, another west coast sealoch. These authors found it possible to make some correction for resuspended material. Extrapolating this rate over the whole loch for the year gives 650 tonnes/yr. It is difficult to assign an error range to this estimate so our use of the value 700 $\pm$ 100 tonnes/yr is somewhat arbitrary, but takes into account the fact that if the Steele and Baird rate were used a value of 800 tonnes would result.

The results of this budgeting of the loch carbon are summarised in Table 3. The value of 1200 tonnes C/yr. for water column respiration is the result of balancing the input and output totals. A figure based on 5% particulate carbon respired per day is 800 tonnes/yr. Oxygen demand measurements made by Tyler and Tett (unpublished) put the respiration rate at 3500 tonnes per year. However this measurement was made by incubating water samples in small bottles so surface effects might be expected to inflate this result.

TABLE 3 Summary of Organic Carbon Budget for Loch Creran

Source/Sink	Input C[a]	Output C[a]	Possible Error[a]
Rivers	1100		$\pm$ 150
Phytoplankton	800		$\pm$ 150
Macro-algae	300		$\pm$ 100
Seaweed Plant	800		$\pm$ 100
Sediment		700	$\pm$ 100
Sea		1200	$\pm$ 400
Respiration		1100	$\pm$ 1000

[a]Figures in tonnes carbon per year.

A Model of the Carbon Flux through Loch Creran

The existence of two distinct types of water in each of the basins of the

loch and in the sea outside, based on the TOC measurements, is in keeping with the two layer circulation observed in fjordic lochs (Edwards and Edelsten, 1976). We have therefore constructed a mathematical model of the loch in which there are six compartments representing the surface and deep waters in the Lynn of Lorne, the main basin and the upper basin. A schematic diagram of this model is shown in Fig. 2. The rates of water movement represented by the

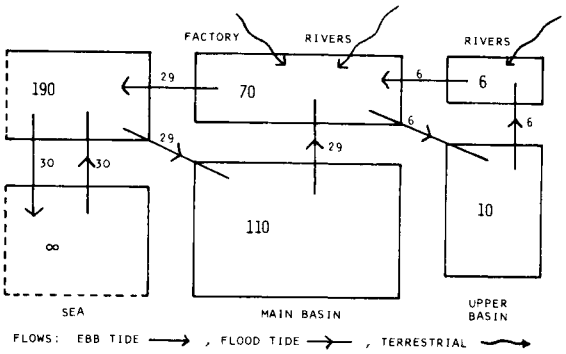

Fig. 2. Box model of Loch Creran. Volume units 10^6 m^3 (numbers inside boxes). Flow units 10^6 m^3/day (numbers by arrows).

arrows were taken as follows. The tidal volume is the area of the basin times the mean tidal range. The fresh water input is from the gauged flow of the River Creran and the area of the catchments as described above. The volumes of the compartments are calculated from the depth of the sills at low water and the areas of the basins. The volume of the deep water of the Lynn of Lorne is taken to be infinite. The volume of the Lynn surface water compartment is calculated from the products of the average depth of water to the halocline and the area of sea over which the effects of Loch Creran are still detectable. In the model the inputs of fresh water are perfectly mixed with the loch water. Water that passes from the surface compartment of the upper basin to the main basin is mixed only to the extent of 75%. This mixing coefficient is based on observations made during a 24 hour monitoring of salinity at the sill (Grantham and Tett, unpublished). During the flood tide the surface water from the Lynn of Lorne passes into the deep compartment of the main basin and the surface water of the main basin enters the deep compartment of the upper basin. Mixing between deep and surface compartments takes place during the flood tide. On the ebb tide surface water flows out of both basins. The fresh water input is divided equally between the ebb and the flood tide. The resolution of the model is one tidal cycle. Using mean values for the daily river flow, the tidal volume and the TOC levels in the rivers and in the deep water in the Lynn of Lorne and the appropriate equations for the mixing of water from the compartments, the model was run for 30 simulated days using a routine written for the Hewlett-Packard 9825 computer. The model reached a steady state after one simulated week. The final TOC concentrations fall short of those actually measured but are related to each other in the same way as the real values. Values for the surface water outside the loch and the deep water in the main basin are equivalent as are those of the surface water of the main basin and the deep water of the upper basin. The TOC values themselves are ranked in the same order as the measured values.

A more elaborate version of the model includes the contributions of the seaweed processing plant, the phytoplankton and the macro-algae. The industrial

input is injected at a constant rate of 1/365 of its annual production per day. The micro- and macro-algal inputs are varied according to their monthly production figures (Johnston, Jones and Hunt, 1977, Tett and Wallis, 1978 and Wood, Tett and Tyler, unpublished). The fresh water input of TOC was varied daily by selecting values from a normally distributed set of values generated by the computer as a function of a pseudo random number generating programme. The simulation was run for consecutive periods corresponding to the sampling intervals used in the field programme. The mean values and variances of the generated TOCs are shown in Table 2. The mean values are somewhat lower than the measured means but again parallel one another in both their ranking and their relationships. The ranking of the variances of the model and actual means are also closely matched. This similarity of behaviour is taken as a sign of the success of the simple box model in mimicking the loch in dilution and discharge of the organic matter entering it.

CONCLUSION

From measurement of TOC at a limited number of stations we were able to construct a useful model of the carbon flux in a fairly complex estuary system. We feel with some further refinements we will be able to use this model to predict the effect of changes in the inputs of carbon on the over-all distribution of TOC.

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Analytical Techniques Used in the Monitoring of Radioactive Discharges from the CEGB Nuclear Power Stations

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ABSTRACT

The Central Electricity Generating Board controls eight operational Nuclear Power Sites in England and Wales. Each station has an authorisation for radioactive discharge which is controlled by the station. In addition samples of the discharge are collected and analysed by one of three laboratories to determine the types of radionuclide discharged and the percentage contribution to the total activity. This paper will describe the current analytical techniques used in the determination of some 30 radionuclides. This includes radiochemical separation of some pure beta emitting nuclides such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{45}Ca, ^{89}Sr, ^{90}Sr, ^{90}Y, ^{91}Y and ^{147}Pm, the separation of ^{51}Cr, ^{55}Fe and ^{63}Ni and automatic gamma-ray spectrometry to evaluate the concentrations of the remaining nuclides.

Emphasis will be placed on the methods employed to ensure correct identification of each nuclide and on the standardisation of chemical and counting methods required to obtain accurate results. Problems associated with the sampling of discharges and the possible loss of activity before analysis has been performed will be discussed.

A comparison of the sum of the individual nuclides with gross activity measurements normally associated with monitoring of discharges will be made.

KEYWORDS

Nuclear Power Sites, liquid radioactive discharge, radiochemistry, gamma-spectromet analytical techniques, standardisation, monitoring.

INTRODUCTION

The Central Electricity Generating Board (CEGB) is currently responsible for the operation of eight gas-cooled Magnox stations and one Advanced Gas-Cooled Reactor (AGR) station in England and Wales. All Stations have an authorisation to discharg liquid radioactive effluent. This authorisation is granted jointly by the Department of the Environment and the Ministry of Agriculture, Fisheries and Food (MAFF) in England and the Welsh Office and MAFF in Wales.

The type of radionuclides and quantity of radioactive liquid effluent can change between the different stations and their potential effect in the local environment

is variable. As a result the authorisation for each station is unique and imposes limits on the amount of radioactivity discharged in any given period and states conditions under which discharges may be made. The Magnox stations at Berkeley, Hinckley Point 'A', Dungeness 'A', Sizewell, Oldbury-on-Severn and Wylfa have a discharge limitation on total activity excluding tritium and on tritium. The remaining two Magnox stations at Bradwell and Trawsfynydd have additional limitations. In the case of Bradwell a limitation is also placed on the discharge of ^{65}Zn due to the presence of oyster beds close to the station discharge point. Trawsfynydd has a limitation on ^{137}Cs as the discharges are made to an inland lake and ^{137}Cs accumulates in fish (mainly trout) caught for local consumption. The AGR station at Hinckley Point 'B' is authorised to discharge total activity excluding tritium, tritium and sulphur -35.

The current authorised discharge limits and the percentage of authorised discharge utilised for the years 1974-76 were published (Groom, 1977). The percentage of authorised discharge utilised was no greater than 80% for any one station in one year. The maximum exposure of an individual expressed as a percentage of the ICRP recommended dose limit was determined to be <0.1% for all stations except Trawsfynydd which gave a mean figure of 10% for the three years reviewed.

In order to determine the radioactive composition of the liquid effluent discharges, samples are taken at the time of discharge by station Health Physics staff. They analyse for total activity excluding tritium, tritium and any other nuclide specified in the authorisation.

In order for MAFF (Dutton, Harvey and Mitchell, 1977) and CEGB to be able to demonstrate that these discharge limits do result in acceptable environmental levels it is necessary to analyse the effluent discharged to obtain a complete radionuclide inventory. This is not possible on every discharge but it is performed on three-monthly bulked samples. The task of analysis is performed by three laboratories, The Central Radiochemical Laboratory (CRL) of the CEGB, the Fisheries Research Laboratory of MAFF and the Laboratory of the Government Chemist. This paper describes the involvement of CRL in this programme and the analytical techniques employed to determine 32 radionuclides likely to be present in liquid effluents.

LIQUID EFFLUENTS

The effluents from stations will contain fission products and activation nuclides arising from the storage of irradiated fuel in water cooling-ponds prior to shipment off-site. In addition the coolant of the reactor becomes contaminated with activation products of the graphite moderator, the coolant and other reactor components. These appear as liquid waste during purification of the coolant gas. All liquid effluents are stored in delay tanks to allow some activity to decay before discharge.

Prior to discharge the contents of the tank are mixed using re-circulation pumps and a representative sample taken typically 1 ml per 1000 litres discharged. These samples are placed in polythene bottles to which 2 litres of 6M nitric acid have been added. The bulk sample for a three month period is sent to one of the analysing laboratories. The use of polythene and the acidification of the effluent ensure that loss of radionuclides from solution by adsorption is minimised. Carriers for certain elements have been used in the past but are not currently necessary if sample is acidified. The use of acid results in loss of carbonate and hence ^{14}C from the solution and a separate non-acidified collection is made for ^{14}C analysis.

ANALYTICAL TECHNIQUES

The list of radionuclides determined in the three-monthly sample of liquid effluent are given in Table 1, giving the half-life of the nuclide and its principal decay modes including that which is used to determine the quantity of radionuclide present in the sample. The majority of nuclides are determined by gamma-spectrometry using a Ge/Li detector but it is necessary to determine eleven radionuclides independently following radiochemical separation and these will be discussed in subsequent subsections. Gross alpha-activity and gross beta-activity are also determined.

TABLE 1 Nuclides Determined In Liquid Effluent.

Nuclide	Half-life	Principal Decay Modes and Abundances (Absolute)
^{3}H	12.35y	Beta, E_{Max} 0.0186 MeV (100%)
^{14}C	5730y	Beta, E_{Max} 0.156 MeV (100%)
^{32}P	14.3d	Beta, E_{Max} 1.710 MeV (100%)
^{35}S	87.2d	Beta, E_{Max} 0.167 MeV (100%)
^{45}Ca	164 d	Beta, E_{Max} 0.257 MeV (100%)
^{51}Cr	27.7d	Electron capture leading to Vanadium X-rays 5 to 6 KeV (22%) Gamma 0.320 MeV (9%)
^{54}Mn	312.5d	Electron capture leading to chromium X-rays 5.5 KeV (25%) Gamma 0.835 MeV (100%)
^{55}Fe	2.7y	Electron capture leading to Manganese X-rays, 5.9 KeV (31.5%)
^{58}Co	70.8d	Positron, E_{Max} 0.475 MeV (15%) Electron capture (85%). Gamma 0.811 MeV (99.4%)
^{59}Fe	44.6d	Beta E_{Max} 0.274 MeV (45.8%) & 0.467 MeV (52.7%) Gamma 0.193 MeV (2.8%) & 1.099 MeV (55.8%)
^{60}Co	5.3y	Beta, E_{Max} 1.48 MeV (0.12%) and 0.314 MeV (99.9%) Gamma, 1.173 (100%) and 1.332 MeV (100%)
^{63}Ni	92.0y	Beta, E_{Max} 0.067 MeV (100%)

TABLE 1 (Cont'd.)

Nuclide	Half-life	Principal Decay Modes and Abundances (Absolute)
^{65}Zn	243.8d	Positron, E_{Max} 0.325 (1.46%) & Electron capture (98.54%) Gamma, 1.115 MeV (49%)
^{89}Sr	50.5d	Beta, E_{Max} 1.463 MeV (100%)
^{90}Sr	28.5y	Beta, E_{Max} 0.546 MeV (100%)
^{90}Y	64.1h	Beta, E_{Max} 2.274 MeV (99.98%)
^{91}Y	58.5d	Beta E_{Max} 1.545 MeV (99.7%)
^{95}Zr	64 d	Beta E_{Max} 0.365 MeV (54.7%) & 0.398 MeV (44.6%) Gamma 0.724 (44.5%) and 0.757 MeV (54.6%)
^{95}Nb	35.1d	Beta E_{Max} 0.160 MeV (99.9%) Gamma 0.766 MeV (99.8%)
^{106}Ru	369d	Beta E_{Max} 0.039 MeV (100%)
^{106}Rh	30.4s	Beta E_{Max} 3.54 MeV (78.9%) Gamma 0.512 MeV (20.6%) and 0.622 MeV (9.9%)
^{110m}Ag	253d	Beta E_{Max} 0.084 MeV (67.6%) and 0.531 MeV (31%) Gamma 0.657 MeV (94.2%), 0.678 MeV (11.1%) 0.706 MeV (16.3%), 0.764 MeV (22.5%) 0.885 MeV (71.7%), 0.937 MeV (34.4%) 1.384 MeV (25.7%) & 1.505 MeV(13.7%)
^{124}Sb	60.2d	Beta E_{Max} 0.61 MeV (52%) and 2.30 MeV (23%) Gamma 0.603 MeV (98.0%), 0.645 MeV (7.2%) 0.723 MeV (11.2%) & 0.968 MeV (1.9%)
^{125}Sb	2.77y	Beta E_{Max} 0.302 MeV (40.2%) + others Gamma 0.321 MeV (0.5%), 0.428 MeV (29.8%) 0.463 MeV (10.4%) 0.601 MeV (17.8%) and 0.608 MeV (4.9%)
^{125m}Te	58d	Internal transition (100%) Gamma 0.108 MeV (0.3%)

TABLE 1 (Cont'd.)

Nuclide	Half-life	Principal Decay Modes and Abundances (Absolute)
^{134}Cs	2.06y	Beta E_{Max} 0.09 MeV (26%) & 0.66 MeV (71.5%) Gamma 0.563 MeV (8.1%),0.569 MeV (14.0%) 0.605 MeV (97.5%)0.796 MeV (85.4%) 0.802 MeV (8.6%), 1.038 MeV(1.0%) 1.168 MeV (2.0%) and 1.365 MeV (3.3%)
^{137}Cs	30.1y	Beta E_{Max} 0.512 MeV (94.6%) Via ^{137m} Ba Gamma 0.662 MeV (85.1%)
^{144}Ce	284d	Beta E_{Max} 0.316 MeV (76.3%) Gamma 0.133 MeV (10.8%)
^{144}Pr	17m	Beta E_{Max} 2.994 MeV (97.75%) Gamma 0.696 MeV (1.53%) & 1.489 MeV (0.28%)
^{147}Pm	2.62y	Beta E_{Max} 0.225 MeV (100%)
^{154}Eu	16.0y	Beta E_{Max} 1.85 MeV (10%) Gamma 0.122 MeV (38%) 0.248 MeV (6.6%) 0.592 MeV (4.6%), 1.274 MeV (37%)
^{155}Eu	1.81y	Beta E_{Max} 0.25 MeV Gamma 0.105 MeV (20%), .087 MeV (32%)

Gamma-spectrometry

Gamma-spectrometry is performed using a 100 cm^3 Ge/Li detection system coupled to a PDP 1105 S Computer. The computer controls eight spectrometers in all including two gamma-spectrometers and accumulates data from the gamma-spectrometers over an energy range 0-2 MeV in 1000 channels. The detector has a resolution of 2 KeV at 1332 KeV.

All analyses are performed using a standard pot filled to a volume of 140 ml. The pot is sealed during counting and a magnetic stirrer employed to ensure that the sample remains homogeneous. The sample is counted typically for 1000 minutes and the spectrum analysed for ^{134}Cs and ^{137}Cs. As the ^{137}Cs is often the major component of liquid effluent it and ^{134}Cs may mask traces of other radio-nuclides present. The caesium is therefore removed in the following way. To 500 ml sample of effluent carriers for all likely elements are added and the solution boiled with 2M nitric acid. After standing any solids are transferred to a counting pot and

the solution passed through a column containing ammonium molybdo-phosphate coated on silica gel which retains the caesium nuclides. The eluate and further 2M nitric acid washings are added to the solids in the counting pot. The pot is then counted as before. A typical spectrum is shown in Fig.1.

Gamma spectra are analysed using the computer. The spectrum analysis programme examines the recorded spectrum and identifies maxima in the count (i.e. detects peaks). Having detected a 'peak' the minima in the count on either side (LBGD and RBGD in Fig.2) are identified and the background continuum under the peak determined by linear-extrapolation between these minima.

EFFLUENT EE/3/75 THROUGH COLUMN

PEAK	LBGD	RBGD	CNTS/MIN	KEV	ISOTP.	NCI/UNIT
0044	0042	0050	00001.4730	0132.1852	CE 144	0000.6837
0157	0152	0159	00000.3730	0320.1381	C& 051	0004.3749
0221	0218	0224	00006.7000	0426.5893	SB 125	0002.6934
0243	0239	0245	00002.1420	0463.1819	SB 125	0002.7066
0272	0267	0279	00001.7220	0511.4176	MC 002	0000.0000
0338	0333	0341	00000.4170	0621.1954	RU 106	0000.8185
0347	0341	0349	00001.6170	0636.1651	S* 125	0002.5093
0362	0356	0365	00001.2940	0661.1146	CS 137	0000.2548
0383	0381	0384	00000.0410	0696.0439	PR 144	0000.6153
0425	0421	0435	00000.2090	0765.9025	NB 095	0000.3295
0443	0440	0453	00000.1140	0795,8419	C+ 134	0000.0258
0466	0461	0469	00000.3210	0834.0978	MN 054	0000.0817
0536	0535	0538	00000.0410	0950.5288	?? +-	
0635	0630	0648	00000.3880	1115.1955	ZN 065	0000.2733
0670	0666	0673	00002.0320	1173.4110	CO 060	0000.5820
0765	0756	0770	00001.8300	1331.4245	CO 060	0000.5854
0843	0837	0852	00000.5240	1461.1619	K 040	0000.0000
0922	0918	0927	00000.1170	1592.5626	TL 208	0000.0000
0981	0975	0983	00000.6210	1690.6973	SB 124	0001.5888

Fig.2 Typical print out of analysis of gamma-spectrum

The background is then subtracted from the integrated counts of all channels between and including the minima. The significance of a peak is determined by comparing the peak height of the peak with the standard deviation of the count of the two minima. The programme can be set to reject peaks which have peak height less than 1, 2, 3 etc. standard deviations of the background counts. From previous calibration of the detector the energy corresponding to the peak position can also be determined and this value is compared with a computer library of 200 gamma-energies greater than 70 KeV including all those listed in Table 1 to identify the radionuclide. The energy must be within $\pm$ 2 KeV for positive identification. The quantity of each radionuclide present is calculated from predetermined counting efficiencies and values for the abundances of each decay mode. The gamma detector is standardised with fifteen radionuclides covering the whole energy range and an energy versus efficiency graph is drawn. Efficiencies at intermediate energies are obtained from a polynomial function fitted to the calibration points. All results are also corrected for decay to the mid-time of the 3 month discharge period. A typical computer print out is depicted in Fig.2. Some of the detector details are omitted.

Many nuclides are identified by more than one peak and a concentration for each peak is calculated. The operator can choose the most abundant peak, the peak with least

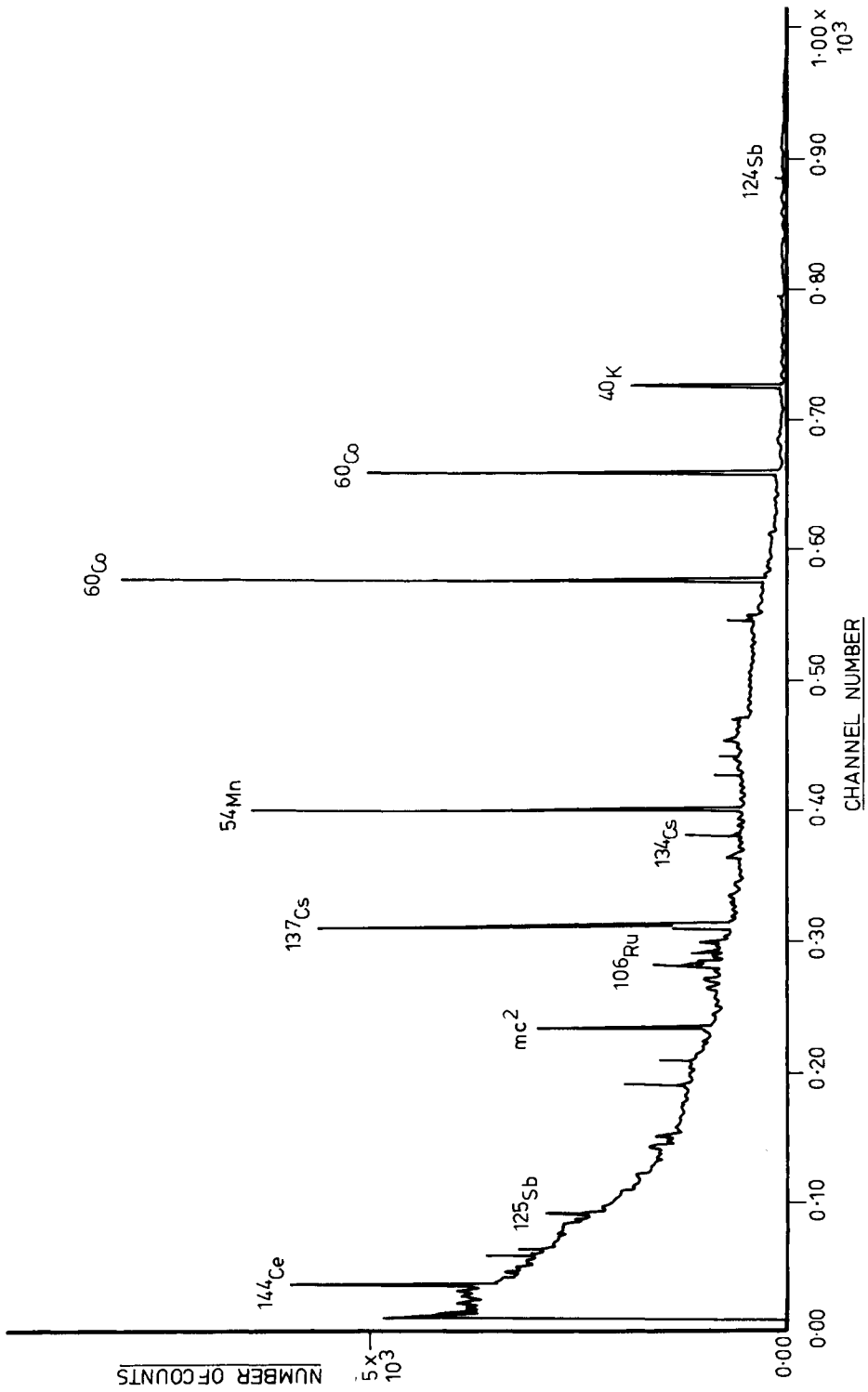

FIGURE 1 TYPICAL GAMMA SPECTRUM OF LIQUID EFFLUENT AFTER REMOVAL OF MOST OF THE CAESIUM NUCLIDES

interference or a mean of results from all the peaks. Peaks which are known to have interferences or nuclides which have similar energies are marked * and & respectively in the print-out. An example of this is the peak for 320 KeV gamma-radiation from ^{51}Cr which coincides with the 320 KeV gamma-radiation from ^{125}Sb. If other peaks confirm the presence of ^{125}Sb then a correction must be applied to the ^{51}Cr determination. Peaks which arise and cannot be identified from the computer library will be denoted by ?? and further identification will be required.

Tritium

Tritium is separated from the sample matrix by distillation (Loveridge). 25 ml of effluent are distilled in the presence of hold back carrier for iodine and 2 ml of the distillate mixed with 10 ml of NE 250 scintillator and counted in a liquid scintillation counter. A standard sample of tritiated water and a blank distilled water are also counted in the same way. The counting efficiency is determined from the measured standard and is of the order of 35%. In addition the sample is counted in an energy channel immediately above that of tritium to show that no higher energy beta-radiation is present.

Carbon-14

The analysis for ^{14}C is performed on the unacidified liquid effluent sample. The method (Gay and Owers, 1967) involves the separation of ^{14}C from the other beta-emitting nuclides and in particular ^{35}S which has a very similar energy to that of ^{14}C (see Table 1). The separation is performed in a closed system to eliminate contamination by atmospheric CO_2. An aliquot of liquid effluent is treated with hydrogen peroxide to ensure ^{14}C and ^{35}S present as carbonate and sulphate respectively. Perchloric acid is then added to release ^{14}C as CO_2 which is carried over using nitrogen gas to a NaOH bubbler which absorbs the carbon dioxide. Barium chloride is added to precipitate barium carbonate which is washed, dried and mixed with 4 g of NE 221 scintillant and counted.

The standardisation of the method is obtained by repeating the duplicate analyses using further portions of liquid effluent which have been dosed with a known amount of ^{14}C tracer. This not only measures the efficiency of counting but also checks on the percentage collection of CO_2 in the sodium hydroxide. Background samples are prepared from pure inactive barium carbonate. Samples, standards and backgrounds are counted after light adaption in a liquid scintillation counter. After background subtraction the amount of ^{14}C present in the sample taken can be determined by comparison with the count rate of the standard and a knowledge of its specific activity.

Phosphorus -32

This analysis is not performed on the three-monthly samples due to its short half-life of only 14.3d. It has been applied to samples analysed within a few weeks of discharge.

The method used involves the separation of caesium by precipitation with chloroplatinic acid. The phosphorus is precipitated in acid solution leaving most of the other radionuclides in solution. Radionuclide contamination by antimony is removed by precipitating antimony as the sulphide. Following a ferric hydroxide scavenge to remove the last traces of other radionuclides, the phosphorus is precipitated as magnesium ammonium phosphate. The precipitate is weighed, dissolved in hydrochloric acid and counted for Cerenkov radiation using a liquid scintillation counter. Calibration is performed by analysing a liquid effluent sample dosed with a known amount of ^{32}P. The isotopic purity is checked by recounting after 14 days and comparing the measured activity with that expected for a half-life of 14.3 days.

Sulphur -35

An aliquot of effluent is acidified and hydrogen peroxide added to ensure sulphur present as sulphate. The solution is evaporated to dryness and redissolved in water. Ruthenium is removed by conversion to the ruthenate and extracted into carbon tetrachloride. The aqueous phase is treated with ammonia solution to precipitate iron and antimony. The solution is then passed through a cation exchange column and the eluate collected. Sulphate is now precipitated as barium sulphate which is washed, dried and weighed into a counting vial. 4 g of NE 221 scintillant are added, mixed and counted on a liquid scintillation counter. As for other methods an effluent dosed with ^{35}S is analysed in the same way.

Calcium -45, Strontium -89 and Strontium -90

Calcium and strontium are separated from an aliquot of effluent by precipitation as oxalate. The oxalate is ignited and the residue dissolved in hydrochloric acid. Following the addition of suitable carriers antimony and ruthenium are removed by passing hydrogen sulphide. Calcium and strontium are now separated using fuming nitric acid when strontium precipitates.

The precipitate is dissolved in water and further treated with fuming nitric acid after the addition of calcium carrier. Barium is removed by precipitation as barium chromate. Iron carrier is added and precipitated as the hydroxide when yttrium will coprecipitate. The step will separate ^{90}Y the radioactive daughter of ^{90}Sr from its parent. The time and date of this precipitation must be noted.

The solution containing ^{90}Sr is allowed to stand and the ^{90}Y to grow in. The ^{90}Y is subsequently separated as the hydroxide when the time and date are again noted. The ^{90}Y is counted using a low level beta counter and its purity checked by determining its half-life by recounting after 2-3 days. The quantity of ^{90}Sr present in the initial effluent can be determined from the ^{90}Y count and a knowledge of the $^{90}Sr/^{90}Y$ equilibrium following growth of ^{90}Y and the strontium and yttrium chemical yields for the analytical method. These latter two yields are determined by weighing the final strontium and yttrium precipitates and comparing them with the known weights of strontium and yttrium carriers added. The ^{90}Sr solution left after ^{90}Y has been extracted is precipitated as the carbonate and counted through a ^{90} mg cm $^{-2}$ absorber. From this the ^{89}Sr content of the effluent can be determined. A fuller discussion of these calculations is given elsewhere (Evett and Davies, 1973).

The supernate from the initial fuming nitric acid separation contains the ^{45}Ca. It is further purified by another fuming nitric acid extract and barium is removed following the addition of carrier as barium chromate. Iron and chromium are removed by precipitation as hydroxides. The calcium is finally precipitated as calcium carbonate, washed, dried and weighed into a tared glass scintillation bottle to determine the chemical yield. The initial calcium content of the liquid effluent must be determined separately and allowance made in the calculation of the yield. The carbonate is suspended in NE 221 scintillant and counted in a liquid scintillation counter. A similar weight standard and a blank calcium carbonate source are also counted under similar conditions.

Chromium-51, Iron-55 and Nickel-63

These three nuclides are analysed in a single sample following an initial group separation. Carriers are added for the three elements to be analysed and any other cations likely to be present. Following initial digestion to ensure cations

in solution the group separation is performed according to the scheme in Fig.3.

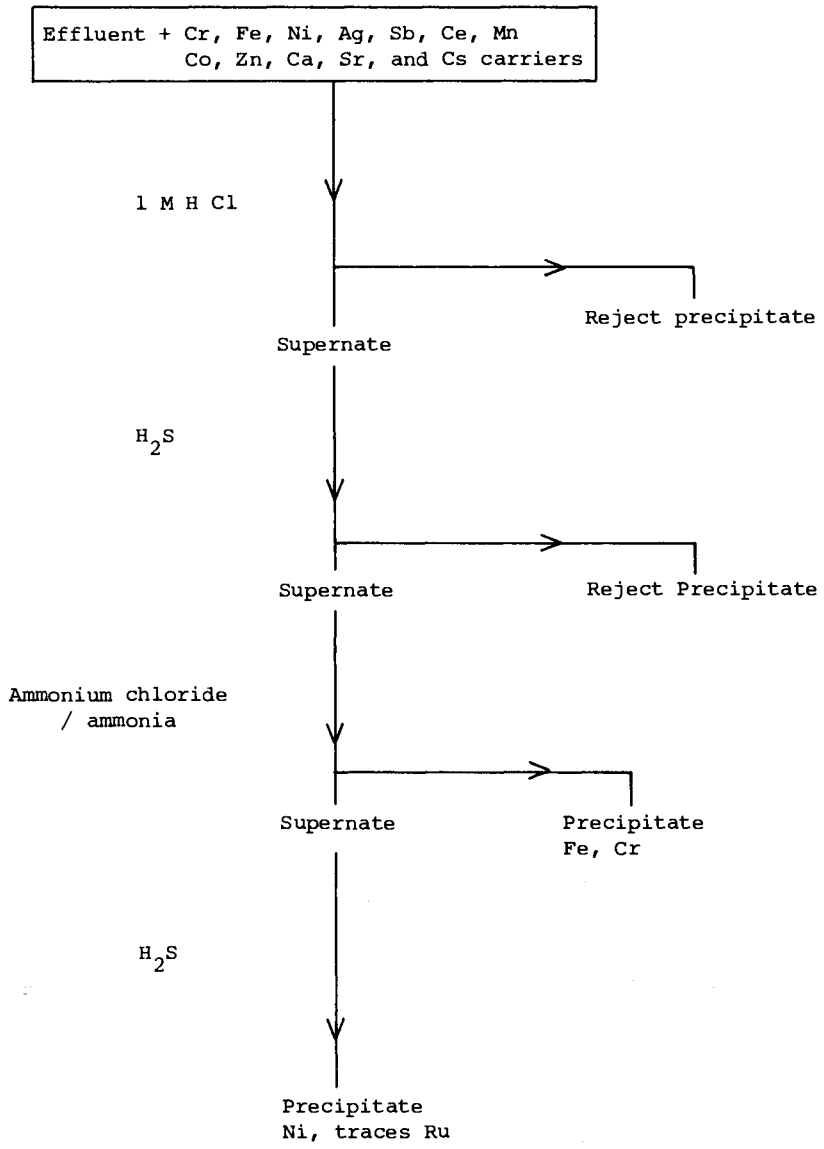

Fig.3 Group separation of Fe, Co and Ni

The iron and chromium precipitate is dissolved in hydrochloric acid and the iron extracted into diethyl ether. The iron is reextracted into water, iron is precipitated and weighed to obtain chemical yield. An allowance for the iron content of original effluent is made. The ^{55}Fe X-ray is counted on a Si/Li detector with a thin beryllium window. A background source and a standard source of known activity and similar weight are also counted.

Under normal circumstances when ^{125}Sb is absent or of very low concentration, the ^{51}Cr may be determined from the gamma spectrum of the original effluent. If ^{125}Sb is present then ^{51}Cr is counted in the aqueous phase of the diethyl ether extraction above. After counting the chromium is precipitated as the acetate, weighed and the chemical yield of chromium determined. The nickel sulphide precipitate is dissolved in concentrated hydrochloric and nitric acids and contaminating manganese removed as the dioxide. The nickel is finally precipitated as nickel dimethyl glyoxime after removal of ruthenium by heating the solution in the presence of 60% perchloric acid. The nickel dimethyl glyoxime is washed and weighed into a glass scintillation vial to determine chemical yield. The complex is destroyed by fuming nitric acid and the solution evaporated to dryness and the residue dissolved in 2 ml water plus a trace hydrochloric acid. The solution is mixed with 5 g of NE 520 solubiliser and 10 ml of NE 233 scintillant and counted on a liquid scintillation counter. A standard and blank source are also prepared and counted.

Yttrium -91 and Promethium -147

To a 100 ml aliquot of liquid effluent calibrated carriers for neodymium, samarium and yttrium are added and also carriers for caesium, strontium and cerium. The rare earths and yttrium are precipitated as hydroxides. The precipitate is dissolved in 6M HNO_3 and zirconium carrier added. Zirconium is precipitated as the iodate after addition of sodium meta-bisulphite and potassium iodate solution. Potassium bromate is added to the supernate and heated to precipitate ceric iodate. The clean-up is completed by further repeated precipitation of hydroxides. The final precipitate is dissolved in 0.1M HNO_3. Yttrium is extracted with di(2 ethyl hexyl) phosphoric acid. This will contain yttrium -90 and yttrium -91. ^{91}Y is counted after ^{90}Y has decayed. The ^{91}Y is confirmed by measurement of its half-life. A yttrium chemical yield is obtained by burning yttrium extract in crucible and weighing as Y_2O_3.

After extraction of yttrium the Pm, Sm and Nb remaining are precipitated as the hydroxides. The ^{147}Pm is determined by measuring count rate with and without a 10 mg cm^{-2} absorber. The percentage transmission is compared with standard sources. As the chemical separation is not good, the sample may be counted on an X-ray detector and presence of ^{154}Eu, ^{155}Eu and ^{144}Ce determined from which corrections can be applied to the beta count. The sample is burnt to obtain the oxides and weighed as Sm_2O_3 and Nb_2O_5 to obtain chemical yield.

Gross beta and alpha activity

A sample of liquid effluent is dried on a stainless steel tray and counted on a gas-flow geiger counter. The standard source used is ^{137}Cs and a background source is also counted. The source weight is limited to 200 - 300 µg to minimise self-absorption of soft beta-emitters such as ^{35}S. The detector has a reasonably constant counting efficiency for beta-energies greater than 0.2 MeV.

Gross alpha-activity is determined by counting a similar dried effluent on a silicon surface barrier detector. The detector produces an alpha-spectrum so that some alpha emitters can be measured individually. The gross alpha-activity is measured relative to ^{239}Pu as standard. Both gross activity figures are recorded at the time of counting. No decay corrections can be made as the nuclides present have a range of half-lives.

CALIBRATION AND STANDARDISATION

All methods involve the use of calibrated nuclide solutions to obtain reliable results, and these are often added to aliquots of the original effluent to measure recovery rates of the separation processes. In addition the separated nuclide samples are also tested to ensure that only the required nuclide is present and being counted. This is achieved in one of several ways:-

a) a check is made on the half-life of the activity measured

b) a determination of the gamma or X-ray energy is made

c) activity is counted in two channels employed on liquid scintillation counters to ensure that no other activity is present.

d) an absorber is employed to distinguish between different beta-energies.

Examples of all these are seen in the methods described.

The methods of analysis are further checked by performing series of intercomparison exercises between the three analytical laboratories and the staff on each nuclear station. These take the form of either all laboratories analysing the same station effluent or analysing mock effluent made up by an independent laboratory. In addition CRL takes every opportunity of participating in IAEA Intercomparison Exercises.

In order to identify any activity unaccounted for the beta-activity associated with each of the gamma-emitting nuclides determined and that of the pure beta-emitters is corrected for decay to the date of the gross beta measurement and integrated. A comparison of the gross beta measured and the integrated beta activity is made and any large discrepancies investigated.

CONCLUSIONS

The use of a complete radionuclide analysis has been described. The results obtained have been totalled for the year and compared with the station returns which are based on analyses at the time of discharge. These have been shown to be between 0.95 and 1.20 of the laboratory determinations. This is regarded as good considering the range of methods employed and the fact that there is a difference in time between analyses which could result in loss of activity from solution by physical and chemical means and certainly by decay of the shorter lived nuclides.

The analysis programme provides confirmation of the station discharge returns and more particularly provides the authorising ministries with more detailed radionuclide concentrations so that the effect of individual nuclides on the environment can be considered, and the presence of new nuclides or change in nuclide composition can be immediately detected.

The results of intercomparisons are good and confirms confidence in the analytical data produced for the discharge of liquid effluent from the nuclear power stations in England and Wales.

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Activation Analysis of As, Hg and Se in Some Marine Organisms

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ABSTRACT

A scheme for the analysis of As, Hg and Se in biological materials using neutron activation and radiochemical separation procedures is fully described. The reliability of the entire process was checked with independent methods and then applied to investigate the trace elements contents in some marine organisms. Results are reported and discussed. The found values suggest some correlations for the Hg and Se contents, while the As content seems to be rather independent. The found absolute levels give some evidence of local pollution problems.

KEYWORDS: activation analysis; Arsenic content; fish tissue; marine pollution; Mercury content; radiochemical separations; Selenium content.

INTRODUCTION

Besides the analytical importance of mercury determination in marine pollution, increasing interest in other metals is becoming more and more evident. Recently arsenic and selenium have come into focus as marine pollutants and their analytical determination is becoming important to investigate the possible correlation with other metals. Correlations among trace elements may give more insight into the origin, uptake, metabolic pathway, function and possibly synergic and antagonistic effects (Groth, Stettler, MacKay, 1976). For this purpose neutron activation analysis is one of the most suitable method affording multielemental analysis without the sample contamination problems affecting other techniques (Tjioe, de Goeij, Houtman, 1976).

Work done under a CNR grant from the task project "Oceanography: Heavy metals pollution control".

This paper describes a chemical separation flow involving the samples dissolution in a high pressure decomposition vessel and a chemical separation of the elements of interest on an ion exchanger columns system.

EXPERIMENTAL

Sampling and Storage

Tuna samples were obtained from fishing vessels operating in the Ligurian - -Sardinian Sea. Mullus barbatus and nephrops norvegicus samples were obtained from selected "hot spot" points of the Tyrrenian Sea. The samples were deep frozen at - 20°C individually sealed in poliethylene bags.

Sample Preparation

Although neutron activation analysis is a potential blank - free technique, special attention must be given to sample handling prior to activation in order to avoid contaminations (Guinn, Hoste, 1978).

Metallic objects were avoided as much as possible and also sample handling utensils made of plastic, which not always prove to be adequate (Bernhard, 1976). We handled the frozen sample only with disposable quartz tubes as a punch. The quartz irradiation tubes were throughly cleaned before use, rinsed with deionized water, dried sealed at one end and heated at red heat. Samples of 500 to 1000 mg (wet weight), punched with disposable quartz punch from the frozen material, were transferred in the silica vials and immediately sealed.

Irradiation Procedure

Neutron irradiations were made in the Triga reactor of the Pavia University at a flux of about 9×10^{12} n cm^{-2} sec^{-1}. The irradiation time was limited to 20 hours due to the pressure build-up in the quartz vials caused by radiolysis of the biological materials. A limited pressure build-up in the vials is recommended not only for safety purposes but also to minimize the loss of irradiated materials when the vials are opened.

Poliethylene vials were avoided due to the high losses experimented for Hg, Se and As in this material (Bate, 1971).

Standard reference materials as NBS-RM canned tuna fish were irradiated together with the actual samples (Orvini, Gills, LaFleur, 1974).

Radiochemical Separations

The irradiated samples were allowed to cool for three days, obtaining a substantial attenuation of the radiation level mainly due to ^{24}Na. Before opening the vials, the outside contamination is removed by keeping the vials in nitric

acid for 10 minutes, and rinsing twice with water. Each vial was then cooled in liquid nitrogen and crushed in a poliethylene container. The sample and the quartz splinters were transferred to the Teflon lined high pressure decomposition vessel together with 3 ml of concentrated nitric acid and 1 ml of sulfuric acid (Bernas, 1976). The decomposition vessels were kept to 130°C for one hour in a thermostatic oven and then cooled at room temperature. The vessels were then opened and the solution brought near dryness by mild warming (less than 60°C) on electric heater. A few drops of hydrogen peroxide were added to volatilize bromine. The samples were then recovered with 10 ml of a 3 M HCl solution and transferred directly on a ion exchange column system consisting of a tin dioxide (TDO) column connected in sequence with a column of Dowex ion exchange resin 1 x 8, 100 - 200 mesh. Each column had an internal diameter of 10 mm and an overall lenght of 4 cm. The system was washed with a 5 bed portion of 3 M HCl.

The first column retains arsenic and selenium, while the second retains all the mercury present in the dissolved solution. The columns were then dismantled and gamma counted on a Ge(Li) detector for the quantitative evaluations. The entire process was thorough checked by duplicating some determinations using specific separations for arsenic, mercury and selenium. Arsenic was evaluated by a highly selective method based on arsine generation and retention on a $AgNO_3$ trap. (Orvini, Delfanti, 1978). The results, as shown in Table 1, confirm the reliability of the method which is simple and accurate for this element.

TABLE 1 Method Reliability Check for As

Sample	As content (µg/g wet weight)	
	TDO	AsH_3
Thunnus		
1	0.27	0.26
2	0.53	0.54
3	1.15	1.20
4	1.12	1.19
5	1.31	1.24
6	0.71	0.69

The reliability of the method for Hg and Se determinations was checked using a specific separation of these elements on a powdered copper column (Meloni and others, 1976, 1977). The results, as reported in Table 2, confirm the accuracy of the method, which was then applied on a routine basis to many mari-

ne organisms analysis.

TABLE 2 Method reliability check for Hg and Se

Sample	Hg content (μg/g wet weight) Dowex	Copper	Se content (μg/g wet weight) TDO	Copper
Thunnus				
1	0.43	0.40	0.66	0.62
2	0.53	0.54	0.80	0.80
3	0.75	0.76	0.87	0.88
Mullus				
1	2.62	2.65	0.80	0.89
2	2.04	2.05	0.50	0.46
3	7.55	7.33	1.19	1.17
Nephrops				
1	1.48	1.50	1.28	1.30
2	2.47	2.30	2.60	2.60
3	2.23	2.08	2.09	2.11

Gamma-Spectrum Evaluation

The 561 KeV from ^{76}As and the 136 KeV from ^{65}Se gamma rays were used for the evaluation of arsenic and selenium content. Due to the fact that on the TDO columns also Sb and few others elements remain together with As and Se, the use of a good Ge(Li) detector is compulsory, being necessary to discriminate the 561 KeV gamma ray of ^{76}As from the 564 KeV of ^{122}Sb.

For the mercury evaluation on the Dowex columns, the 279 KeV gamma ray from ^{203}Hg was utilized.

RESULTS AND DISCUSSION

The described method was applied to the determination of selenium, mercury and arsenic in some marine organisms.

The results are reported in Table 3. The values reported for thunnus thinnus confirm the well known situation of mercury contamination in mediterranean

tuna as described by Cumont and others (1972).

TABLE 3 Se, Hg and As Trace Content in Some Marine Organisms (μg/g wet weight)

Sample	Se	Hg	As
Thunnus			
1	0.66	0.43	0.27
2	0.80	0.44	1.12
3	0.80	0.53	0.53
4	0.81	0.96	1.70
5	0.87	0.75	1.15
6	0.92	0.65	1.55
7	1.01	1.30	0.71
8	1.13	1.95	0.85
9	1.32	2.30	1.31
Mullus			
1	0.32	0.95	8.20
2	0.36	0.60	31.10
3	0.41	1.12	39.85
4	0.65	7.13	68.63
5	0.81	1.76	52.00
6	0.90	8.59	84.50
7	1.19	7.55	64.10
Nephrops			
1	0.70	0.74	132.50
2	0.75	1.58	112.90
3	0.80	0.34	55.76
4	1.10	0.94	119.40
5	1.16	1.19	119.90
6	1.28	1.48	25.30
7	1.30	0.60	60.27
8	1.50	0.81	18.46
9	2.09	2.23	133.50
10	2.60	2.47	69.52

Apparently (Fig. 1), no close intercorrelation exists among the three metal contents, which are in the same order of magnitude.

For the mullus barbatus samples collected in "hot spot" points for mercury pollution, the values for mercury are very high although unexceptional on respect to some peak values reported by Renzoni (1977) for the same fish in the same

collecting area. Considerable variations exist between individuals, especially for mercury and arsenic, which vary by a factor of 8 and 10 respectively. The values for arsenic and selenium are in the range of the ones reported by Robertson and Perkins (1972) for similar pelagic fish. A similar accumulation trend is evident for the three elements as shown in Fig. 2. The ratio between mercury and selenium seems not to be stoichiometric confirming the results reported by Koeman and others (1975).

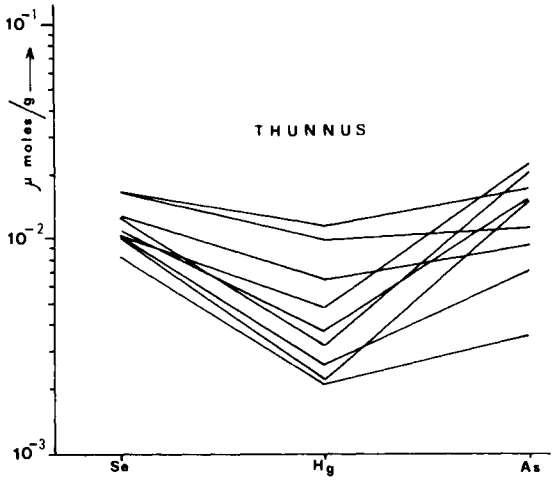

Fig. 1. Se, Hg and As content in Thunnus Thinnus

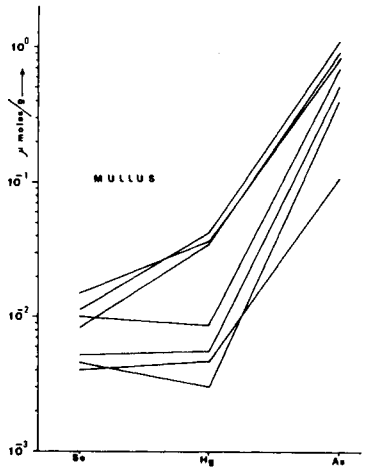

Fig. 2. Se, Hg and As content in Mullus barbatus

The atomic ratio of arsenic to selenium and mercury is very high, reaching in some cases the values of 100.

The mercury and selenium contents of the bottom feeding scampi (Nephrops Norvegicus) collected in "hot spot" points for mercury pollution are very similar to the only reported values for this organism, them also collected in a very polluted area (Kosta and others, 1978). The arsenic values are higher than those in the pelagic fishes thunnus thinnus and mullus barbatus, although the absolute values appear to be unexceptional in comparison with some values reported by Robertson and Perkins (1972) for bottom feeding animals. The atomic ratio of arsenic to selenium and mercury is very high, reaching in some cases the values of 100 as shown in Fig. 3. Although all the three species monitored concentrate mercury, levels in mullus are considerably higher than in thunnus and nephrops.

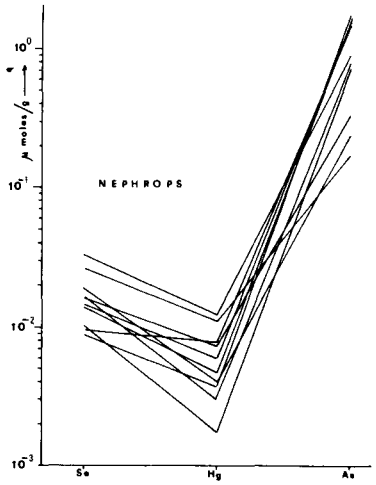

Fig. 3. Se, Hg and As content in Nephrops

These results support the feeling that correlation studies of the accumulation trends for many different elements in marine organisms are worthwhile and that nuclear activation analysis offers a reliable method to this aim being a multielemental technique.

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Application of Ion-exchange Techniques to the Analysis of Natural Waters for Toxic Metals

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ABSTRACT

A discussion of methods currently employed at the Institute for Analytical Chemistry of the University of Vienna, to determine Pb, Cd, Zn, Cu, Tl, Be, Co, Mn, Mo, V, U and Th in natural waters is presented. Most of these techniques are based on anion-exchange enrichment of the metals directly from the water samples to which suitable reagents such as hydrobromic acid, hydrochloric acid, thiocyanate and citrate have been added to produce anionic complexes of the elements which are readily retained by strongly basic anion exchange resins such as Dowex 1. In some cases also reducing or oxidizing agents must be present to guarantee the quantitative adsorption of the metal to be isolated. Also treated are multistep separations in which ion-exchange is combined with other concentration techniques, such as evaporation and/or liquid-liquid extraction procedures. After ion-exchange concentration the metals are eluted and determined by atomic-absorption spectroscopy, spectrophotometry and fluorimetry. Natural waters that have been analyzed successfully by using the described techniques include drinking waters, mineral waters, hydrothermal waters, river waters, sea-water and oil-field waters.

KEYWORDS

Ion-exchange, separation methods, toxic metals, analysis of natural waters.

INTRODUCTION

Quantitative determinations of trace quantities of toxic metals in liquid environmental samples, as for example natural waters, are gaining increased importance with growing interest in pollution of the aquatic environment with elements such as lead, cadmium and mercury as well as with radioactive species of which uranium and thorium are representative elements. For these assays, analytical techniques based on atomic-absorption spectrophotometry, emission spectroscopy, fluorimetry, and spectrophotometry are often employed, especially in combination with preconcentration methods such as those based on ion-exchange and liquid extraction, so that improved detection limits and accuracy can be achieved. Thus, the determination of toxic metals in natural waters is in many cases preceded by some chemical operations with the purpose of concentrating the metal in minimum volume. For this preconcentration, ion-exchange techniques can be used very effectively and the ion-exchange materials most frequently employed in my laboratory are strongly basic anion exchange resins.

DISCUSSION

The principles on which several of these preconcentration techniques are based will now be presented in tabular form, to illustrate most clearly the scope of the ion-exchange methods. Also treated will be multistep separations in which ion-exchange is combined with other concentration techniques, such as evaporation and/or liquid-liquid extraction procedures, which are commonly employed to preconcentrate trace constituents of natural waters.

TABLE 1. Separation and concentration of toxic metals by strongly basic anion exchange resins (Without preliminary use of other preconcentration procedures)

A. Hydrobromic and hydrochloric acid systems

A.1. Cadmium, lead and copper in natural non-saline waters (Korkisch and Sorio, 1975a)

Principle of analysis: Adsorption of Pb(II), Cd(II) and Cu(I) on a 4 g column of Dowex 1, X8 (bromide form) or Lewatite 5080 (bromide form) from a maximum of 500 ml of the sample made ca. 0.15 M in HBr and containing ascorbic acid. Cd, Pb and Cu are eluted with 1M HNO_3 and determined by atomic-absorption spectrophotometry.

Remarks: From the values of the distribution coefficients shown in the table below it is seen that Cd(II), Pb(II) and Cu(I) are strongly adsorbed on the resin as anionic bromo complexes. Cu(II) is not adsorbed so that it must be reduced with ascorbic acid before the acidified sample solution is passed through the ion-exchange column. Not adsorbed on the resin from 0.15 M HBr

Distribution coefficients in 0.15 M HBr (1 g Dowex 1; 1 mg load)

Ion	In presence of ascorbic acid 5 g /liter	In absence of ascorbic acid
Cd(II)	9.1×10^3	9.1×10^3
Pb(II)	1.4×10^3	1.4×10^3
Cu(I;II)	3.3×10^3	<1

are all major and minor cationic constituents of natural waters. Large amounts of sulfate and chloride interfere so that the method is not applicable to the analysis of sea-water, mineral waters and other highly saline waters. Quantitative preconcentration is achieved for both ppb and ppm quantities of Cd, Pb and Cu.

A.2 Lead in river- and drinking waters (Korkisch and Sorio, 1975b)

Principle of analysis: Adsorption of Pb(II) on a 4 g column of Dowex 1, X8 (bromide form) from a maximum of 1 liter of the sample made ca. 0.15 M in HBr. Pb is eluted with 6 M HCl

and determined by atomic-absorption spectroscopy or spectrophotometrically using the dithizone method.

Remarks: On elution of Pb with 6 M HCl coadsorbed Cd is not coeluted so that only lead is found in the eluate. Further details are presented in section A1. of this TABLE 1.

A.3. Thallium in drinking-, mineral-, hydrothermal-, sea- and oil-field waters (Korkisch and Steffan, 1978)

Principle of analysis: Adsorption of Tl(III) on a 4 g column of Dowex 1, X8 (bromide or chloride form) from the water sample made ca. 0.15 M in HBr and containing elemental bromine. Tl is eluted with ca. 3% aqueous solution of SO_2 and determined by atomic-absorption spectrophotometry.

Remarks: In 0.15M HBr containing bromine a weight distribution coefficient of 17×10^3 was measured for Tl(III). Not adsorbed on the resin from 0.15 M HBr + Br_2 are all major and minor cationic constituents of natural waters. Among the trace elements Pb, Cd and Bi are coadsorbed with Tl. In the absence of Br_2, thallium is not retained by the resin because Tl(I) does not form an adsorbable anionic bromo complex. Thus, when treating the resin on which Tl is adsorbed as bromo thallate e.g. $TlBr_4^-$ with sulphurous acid this element is reduced to the monovalent state and as such passes into the eluate. The amount of bromine to be added to the sample solution should not exceed that contained in 10 ml of saturated bromine water irrespective of the volume of the water sample subjected to analysis. Since very large amounts of sulfate and chloride do not interfere with the adsorption of Tl(III) the method can be applied to the analysis of all natural waters.

A.4. Cadmium in tap-, river- and subterranean waters (Korkisch and Dimitriadis, 1973a)

Principle of analysis: Adsorption of Cd on a 4 g column of Dowex 1, X8 (bromide or chloride form) from 1 liter of the sample made 1.0 M in HBr or 1.2 M in HCl. Coadsorbed Zn is removed with 0.15 M HBr and Cd is eluted with 2 M HNO_3. In the eluate Cd is determined spectrophotometrically using the dithizone method.

Remarks: In 1.0 HBr and 1.2 M HCl weight distribution

coefficients for Cd of $2x10^4$ and 1646 respectively were measured. For Zn the distribution coefficients were found to be 100 and $>10^3$ at these two acidities. However, in 0.15 M HBr the coefficient is only 3 so that this acid is a very effective eluent for zinc.

A.5. Copper in river- and drinking waters (Korkisch, Gödl and Gross, 1975b)

Principle of analysis: Adsorption of Cu(I) on a 4 g column of Dowex 1, X8 (chloride form) from a maximum of 200 ml of the sample made ca. 0.1 M in HCl and containing ascorbic acid. Cu is eluted with 1M HNO_3 and determined by atomic-absorption spectrophotometry.

Remarks: In 0.1M HCl containing 5 g of ascorbic acid/liter a distribution coefficient of 480 was measured for copper which is adsorbed on the resin as an anionic chloro complex. In this complex copper is present in the monovalent oxidation state. In the absence of ascorbic acid copper is not retained by the anion exchanger. Not adsorbed on the resin from 0.1 M HCl + ascorbic acid are all major and minor cationic constituents of natural waters. Quantitative preconcentration is achieved for both ppb and ppm quantities of Cu. Larger amounts of chloride and sulfate interfere; hence the technique cannot be used for the analysis of highly saline waters such as mineral waters or sea-water.

B. Thiocyanate systems

B.1. Cadmium, zinc, cobalt, and uranium in river-, tap-, sea- and other natural waters including oil-field waters (Korkisch and Gödl, 1974a, 1974b; Korkisch, Gödl and Gross, 1975a; Korkisch and Steffan, 1975)

Principle of analysis: Adsorption of Cd(II), Zn(II), Co(II), and UO_2(II) on a 4 g column of Dowex 1, X8 (SCN^- form) from the sample made ca. 0.1 M in HCl and containing KSCN (10 or 20 g/liter). To remove SCN^- and coadsorbed metals such as Fe the resin is washed with a 5:4:1 mixture of tetrahydrofuran, methyl glycol and 6 M HCl. Then Co, U, Zn, and Cd are eluted with 6 M HCl, 1M HCl, 0.15 M HBr and 2 M HNO_3 respectively. Co,

Cd and Zn are determined by atomic-absorption spectroscopy or spectrophotometrically (Co and Cd)(nitroso-R-salt and dithizone methods) while U is measured fluorimetrically or by means of the spectrophotometric arsenazo III method.

Remarks: In the presence of 20 g KSCN/liter weight distribution coefficients of 760, 28×10^3, 3.3×10^3 and 85×10^3 were measured for Cd, Zn, Co and U respectively. This means that all four elements are very strongly retained by the resin as anionic thiocyanate complexes. Ascorbic acid is added to the samples to reduce iron to the non-adsorbable divalent oxidation state. In the mixture consisting of 50% tetrahydrofuran, 40% methyl glycol (= monomethyl ether of ethylene glycol) and 10% 6 M HCl the distribution coefficients of Cd, Zn, Co and U have values of $>10^2$, $>10^2$, $>10^2$ and $>10^3$ respectively so that when using this washing solution these metals are not removed from the resin. The method is very well suited especially for the isolation of uranium of which from ppb to mg-amounts can be preconcentrated from natural waters of all kinds.

B.2. Molybdenum and vanadium in river- and sea- waters (Weiss and others, 1977)

Principle of analysis: Molybdenum: Adsorption of Mo on a 4 g column of Dowex 1, X8 (SCN^- form) from the sample made ca. 0.1 M in HCl and containing KSCN (10 g/liter) and ascorbic acid (5 g/liter). Mo is eluted with 2 M $HClO_4$-1 M HCl and determined by atomic-absorption spectrophotometry.

Vanadium: Adsorption of V under the same conditions as described above for Mo. V is eluted with a 9:1 mixture of methanol and 6 M HCl and determined by atomic-absorption spectrophotometry.

Remarks: Under the conditions outlined above for the adsorption of the anionic thiocyanate complexes of Mo and V weight distribution coefficients of $>5 \times 10^3$ and $>10^4$ respectively were measured for these two elements. Ascorbic acid is added to the acidified water samples to facilitate the reduction of Mo and V to valency states which react readily with thiocyanate ion to form complexes which are retained by the resin. Coadsorbed with Mo and V are the thiocyanate complexes of U, Co, Cd, Zn, Cu, Hg,

and Fe(III). Only part of the Mo is co-eluted with the V by the methanol-HCl. All other elements are strongly retained because they form stable anionic chloride complexes under these conditions of elution. Before the elution of Mo with 2 M $HClO_4$ - 1 M HCl it is recommended to wash the resin bed with distilled water and 6 M HCl. Prior to the elution of V only distilled water is used to wash the ion exchanger.

C. Citrate systems

C.1. Uranium, thorium, vanadium, and molybdenum in tap-, mineral-, and river waters (Korkisch and Krivanec, 1976a, 1976b)

Principle of analysis: Uranium and thorium: Adsorption of UO_2(II) and Th(IV) on a 4 g column of Dowex 1, X8 (citrate form) from 1 liter of the sample of pH 3 containing 10 g of citric acid, 3 g of sodium citrate and 2 g of ascorbic acid. Th is eluted with 8 M HCl and separated from co-eluted substances by anion exchange in 8 M HNO_3 medium on a separate 2 g column of the same resin (nitrate form). Fe is removed by washing the 4 g resin bed with a 1:8:1 mixture of hexone (isobutyl methyl ketone), acetone and 1 M HCl and then U is eluted with 1M HCl and determined fluorimetrically. From the 2 g column Th is removed by means of 6 M HCl and is determined spectrophotometrically using the arsenazo III method.

Vanadium and molybdenum: Adsorption of V and Mo on a 4 g column of Dowex 1, X8 (citrate form) from a maximum of 5 liters of the sample of pH 3 containing citric acid, sodium citrate and ascorbic acid. V is eluted with 6 M HCl and subsequently Mo is recovered with 2 M $HClO_4$ - 1 M HCl. In the eluates V and Mo are determined by atomic-absorption spectrophotometry.

Remarks: Under the conditions outlined above for the adsorption of anionic citrate complexes of U, Th, V, and Mo weight distribution coefficients of 4×10^4, 1.6×10^4, 5×10^3, and 5×10^3 respectively were measured for these four elements. Large amounts of sulfate and chloride interfere with the adsorption of thorium.

TABLE 2. Separation and concentration of toxic metals by strongly basic anion exchange resins after preliminary application of other preconcentration procedures

A. Preconcentration by evaporation

A.1. Uranium in sea-water and other natural waters (Korkisch and Steffan, 1972)

Principle of analysis: After evaporation of the sample UO_2(II) is adsorbed on a column of Dowex 1, X8 (Cl^- form) from 50% tetrahydrofuran - 40% methyl glycol and 10% 6 M HCl. Following a washing step with aqueous 6 M HCl, U is eluted with 1 M HCl and determined fluorimetrically.

Remarks: In place of the mixture of tetrahydrofuran, methyl glycol and 6 M HCl, the sorption solution may consist of 90% methyl glycol and 10% 6 M HCl. In this case, however, the column has to be washed with a solution consisting of 80% methyl glycol and 20% 3 M HCl to remove coadsorbed elements e.g. Co, Cu and Mn which interfere with the final determination of U after its elution with 1 M HCl.

A.2. Thorium in tap- and river waters (Korkisch and Dimitriadis, 1973b)

Principle of analysis: The sample (1 liter or more) is acidified with nitric acid and evaporated to dryness. The residue is taken up in 8 M HNO_3 and Th is adsorbed on a 4 g column of Dowex 1, X8 (NO_3^- form). Thorium is eluted with 6 M HCl and determined spectrophotometrically using the arsenazo III method.

Remarks: From 8 M HNO_3 thorium is adsorbed as a negatively charged nitrate complex of the formula $[Th(NO_3)_6]^{2-}$. Under these conditions a weight distribution coefficient of 300 was measured for Th while Ca, Mg and all other major and minor constituents of natural waters are not retained by the resin.

B. Preconcentration by liquid-liquid extraction

B.1. Cadmium, lead, zinc, copper, cobalt, manganese, and uranium in non-saline waters, snow and sea-water (Korkisch and Sorio, 1975c)

Principle of analysis: Cd, Pb, Zn, Cu, Co, Mn and U are extracted as diethyldithiocarbamates with 2:5 acetone-chloroform at pH 5. Then, these elements are adsorbed on a 4 g column of Dowex 1, X8 (Cl^- form) from a 5:4:1 mixture of tetrahydrofuran, methyl glycol and 6 M HCl. Successive elution is effected with 6 M HCl (for Co, Cu, Mn, and Pb), 1 M HCl (for U) and 2 M HNO_3 (for Cd and Zn). In the eluates the elements are determined by atomic-absorption spectrophotometry (except U,which is determined fluorimetrically).

Remarks: Since the rate of decomposition of diethyldithiocarbamate complexes is relatively high at pH 5 the extraction has to be carried out immediately after addition of sodium diethyldithiocarbamate (1 g/liter). After extraction, the extractant is evaporated off and the diethyldithiocarbamates have to be destroyed by evaporation in the presence of tetrahydrofuran, methyl glycol and 6 M HCl. If this is not done the elements are retained incompletely by the anion exchanger. The distribution coefficients listed below were measured in the various media used for adsorption and elution.

Metal ion	Medium			
	Tetrahydrofuran-methyl glycol-HCl	6 M HCl	1M HCl	2 M HNO_3
Cd(II)	705	130	700	<1
Co(II)	6030	<10	<1	<1
Cu(II)	75	12	<1	<1
Mn(II)	200	<10	<1	<1
Pb(II)	250	<10	65	<10
UO_2(II)	3510	250	<1	<1
Zn(II)	305	125	195	<1

C. Preconcentration by evaporation and liquid-liquid extraction

C.1. Uranium in natural waters (Korkisch and Koch, 1973)

Principle of analysis: The sample is evaporated to dryness and U is extracted with 0.1 M trioctylphosphine oxide (TOPO) in ethyl ether from 1 M HCl solution containing ascorbic acid.

The extract is treated with methyl glycol and 12 M HCl to make the solvent composition methyl glycol-0.1 M ethereal TOPO-12 M HCl (9:10:1); from this solution U is adsorbed on a column of Dowex 1, X8 (Cl^- form). After washing with methyl glycol - 30% H_2O_2-12 M HCl (18:1:1) and 6 M HCl, U is eluted with 1M HCl and determined fluorimetrically.

Remarks: In the presence of ascorbic acid the co-extraction of Fe is prevented to a large extent.

C.2. Uranium in natural waters (Hecht and others, 1956)

Principle of analysis: The sample is evaporated to dryness and U is extracted with diethyl ether from a nitrate - HNO_3 solution. After removal of the ether, co-extracted elements are separated by adsorption of U on a column of Amberlite IRA-400 (acetate form) from an acetate solution of pH 4.25 - 5.25. U is eluted with 0.1 M HCl and determined polarographically (catalytic nitrate wave).

Remarks: On the anion exchanger, uranium is adsorbed as a negatively charged acetate complex while iron, thorium and many other elements pass into the effluent unadsorbed.

TABLE 3. Separation and concentration of toxic metals by strongly acidic cation exchange resins after preliminary application of other preconcentration procedures

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Preconcentration by liquid-liquid extraction

Beryllium in tap-, mineral-, river- and sea-water samples (Korkisch, Sorio and Steffan, 1976)

Principle of analysis: Be(II)-acetylacetonate is extracted with chloroform at pH 7 in the presence of EDTA. The chloroform extract is mixed in the ratio of 3:6:1 with tetrahydrofuran and methanol containing HNO_3, and passed through a 4 g column of Dowex 50, X8 (hydrogen form). After removal of acetylacetone, chloroform and tetrahydrofuran by washing the resin bed with methanolic-HNO_3, Be is eluted with 6 M HCl and determined by atomic-absorption spectrophotometry.

Remarks: The chloroform extraction of Be-acetylacetonate is carried out in the presence of EDTA to prevent the co-extraction of most of the main and trace constituents of liquid environmental samples. Because of volatility losses of Be-acetylacetonate which were observed on evaporation of the chloroform extract Be is isolated by the cation exchange technique outlined above. In this medium consisting of the chloroform extract, tetrahydrofuran and methanol with an overall acidity of 0.1 M in HNO_3 a distribution coefficient of 580 was measured for the Be. Under the conditions of elution with 6 M HCl this coefficient has a value of <1. Fe and Cu are coeluted with the Be but do not interfere with the atomic-absorption determination of Be.

In conclusion it should be mentioned that most of the methods presented in the TABLES 1-3 can be employed routinely because separations on columns of ion exchange resins can be performed more or less automatically. Thus, numerous water samples can be treated simultaneously, usually involving fewer manipulations than are necessary when using other concentration and separation procedures such as liquid-liquid extraction or coprecipitation. This is especially true for ion exchange processes that are applicable without preconcentration steps, permitting the water sample to be passed through the columns immediately after addition of the required reagent. With these direct methods which were outlined in TABLE 1 the danger of contamination of the sample by extraneous sources is reduced over the solvent extraction or coprecipitation approaches, which generally require more manual operations.

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Optimized Selenite Determination in Environmental Waters by X-ray Fluorescence

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ABSTRACT

A procedure is optimized for the determination of selenite at the sub-ppb level in environmental water samples by energy-dispersive X-ray fluorescence. The preconcentration step involves the reduction of selenite to elemental selenium by l-ascorbic acid, and the subsequent adsorption onto activated carbon. The activated carbon is then filtered off on a Nuclepore membrane and this target is directly measured by X-ray fluorescence. The analytical characteristics of the overall procedure are evaluated.

INTRODUCTION

From an environmental point of view, selenium is a very important element because depending on the concentration level, it exhibits both toxic and essential element characteristics. The concentration of selenium in environmental water samples is usually below 1μg l^{-1}. Few analysis techniques are capable of determining selenium at this level, even in combination with a simple preconcentration technique.
For selenite, probably the most important species of selenium in the environment, Massée, van der Sloot and Das (1977) proposed a neutron activation determination through the reaction $^{74}Se(n, \gamma)\ ^{75}Se$ after an attractive preconcentration step involving reduction with l-ascorbic acid and subsequent adsorption of the elemental selenium onto activated carbon.
In the present work energy-dispersive X-ray fluorescence (XRF), an economic and more readily available analysis technique, is used and the characteristics of the overall procedure are evaluated.

EXPERIMENTAL

Reagents

All reagents were analytical grade. The water used for dilution and standards was deionized and doubly distilled in quartz material. Standard solutions of

selenium were prepared from Titrisol Standards. The activated carbon, a "Baker Analyzed" reagent, was treated with concentrated HF and HCl, washed with water, and dried at 110°C to reduce the trace metal contamination further by a mean factor of five as found by Vanderborght and Van Grieken (1977).

Apparatus

The XRF instrumentation consisted of a Siemens Kristalloflex 2 high voltage power supply and an X-ray tube with tungsten target, a molybdenum secondary fluorescer and filter, a 16-position sample holder, and a Kevex Si(Li) semiconductor detector with related electronics. A 4096-channel analyzer, coupled to a magnetic tape unit recorded the spectra, which were analyzed by a PDP 11/45 computer using a non-linear least-square fitting program, as is published by Van Espen and co-workers (1977). The area under the Se K_α-peak (11.21 keV) is calculated.
Radiotracer experiments were performed by means of a Ge(Li) detector and a multi-channel analyzer. In all experiments ^{75}Se ($T_{1/2}$ = 120 d) of high specific activity was used.

OPTIMIZATION OF THE PROCEDURE FOR X-RAY FLUORESCENCE

Preconcentration Principle.

In aqueous solutions, selenite can be reduced quantitatively to the elemental state by l-ascorbic acid (Fogg and Wilkinson, 1956; Sherrat and Conchie,1969). Selenate is not or very slowly reduced by this reagent. The resulting colloidal elemental selenium can be efficiently collected by adsorption on activated carbon. When the activated carbon suspension is filtered on a Nuclepore membrane (0.45 µm poresize), the resulting homogeneously loaded filter represents an ideal target for XRF.
Using radiotracer experiments and XRF measurements,all of the variable parameters of this analysis procedure were investigated for standard solutions and for tap and sea water samples.

Influence of the pH.

After addition of the radiotracer, 450 mg amounts of ascorbic acid were added to 100 ml sea water samples of a different pH. After 15 minutes, the solutions were passed through a layer of 100 mg of activated carbon.
A constant yield above 90 % was obtained from 10^{-3} N HCl up to 4N HCl. A pH value of 2 was preferred for further work.

Influence of Ascorbic Acid Concentration.

To a 100 ml batch of sea water with radiotracer, varying amounts of ascorbic acid were added. After a 15 minutes equilibration time colloidal elemental selenium was adsorbed on 100 mg AC. Above 2 mg ml^{-1} of ascorbic acid a high and constant yield was observed.
A 3mg ml^{-2} concentration proved to be convenient.
Selenite adsorption on activated carbon in the absence of ascorbic acid was found to be less than 1 % in the 0.2 to 2 ppb range.

Influence of the Reaction Time with l-Ascorbic Acid.

The time between the addition of l-ascorbic acid and the adsorption of colloidal selenium on an activated carbon layer should be at least 10 minutes to obtain high and constant collection efficiencies. When the adsorption step is carried out in an activated carbon suspension, it appears necesarry to stir the solution during the same time after the ascorbic acid addition.

Influence of the Amount of Activated Carbon.

The necessary minimum amount of activated carbon is a function of the selenite concentration in the solutions. Up to a 5 ppb selenite level, a 100 mg quantity of activated carbon appeared sufficient to capture 90 % of the selenium from 1 l of water.

Influence of the Activated Carbon Type and Target Thickness.

The detection limit of the XRF analysis, defined by three times the square root of the background count rate in the Se-K_α energy region, depends on the blank Se level and on the target thickness.
-Two available activated carbon types were compared: a"Baker Analyzed" reagent, purified according to Vanderborght and Van Grieken (1977), and a high-purity charcoal compound, derived from polyvinylidenechloride (Gouman and van der Sloot, 1978).
The selenium content for both products was found to be below 0.6 ppm Se. The former product was preferred for XRF work because its lower Zn-content produced less spectral interference in the Se-K_α energy region.
-Both the background, which is mainly due to incomplete charge collection in the detector of scattered primary X-rays, and the specific Se-K_α signal, increase as a function of the target thickness. Quantitative calculations show that the resulting detection limits for activated carbon target thicknesses of 2, 5, 10, 50, 100 mg cm^{-2} respectively, are in the proportions of 30, 5.7, 3.0, 1.2 and 1, respectively. This suggests a target thickness above 50 mg cm^{-2} for optimal sensitivities.
On the other hand, the Se-K_α absorption corrections increase for this order of target thicknesses as 1.015, 1.03, 1.07, 1.36 and 1.79 respectively; therefore, the uncertainties on this correction and the resulting accuracy deterioration will be important for such activated carbon loads. As a promise 10 mg cm^{-2} activated carbon targets were preferred. Therefore Nuclepore membranes are loaded with the 100 mg of activated carbon filtering in a 9.6 cm^2 Gelman N° 4209 filter holder.

Influence of the Order of Addition of the Reagents.

The order of addition of the different reagents was found not to be critical at the 1 ppb level. Hence it is possible to add the ascorbic acid before, after, as well as together with the activated carbon to a 1 l selenite solution. The most important thing is a contact-time of 15 minutes before filtration of the suspension.

OPTIMAL CONDITIONS FOR SELENITE RECOVERY.

In view of the above experiments the following procedure can be recommended: add to 1 liter of solution 3 g ascorbic acid and 100 mg of the activated carbon. Stir the suspension for at least fifteen minutes and filter off on a Nuclepore membrane with 10 cm^2 active area. Measure the selenium content of the activated carbon loaded filter by energy-dispersive XRF-analysis.

EVALUATION OF THE PROCEDURE.

Recovery Versus Selenite Concentration.

In five series of experiments the collection efficiences for concentrations of selenite were measured either by tracer experiments or by XRF measurements. The results are represented in Table 1.

TABLE 1 : Recovery of selenite as elemental selenium, adsorbed on activated carbon.

Added amount of Se(IV) in $\mu g\ l^{-1}$	Recovered amount of Se, in %	Coefficient of variation per measurement, in %
0.05	70	8
0.1	85	8
0.25	87	11
0.5	85	6
1	89	6
10	70	7
100	65	8

Apparently, the recovery yield is high and practically constant from below 0.1 $\mu g\ l^{-1}$ up to a few $\mu g\ l^{-1}$ i.e. within the usual environmental range. At the high concentration end, the recovery can be improved either by increasing the contact time with the activated carbon (the recovery of 100 $\mu g\ l^{-1}$ selenite raises to 86 % after a 24 h contact) or by increasing the amount of activated carbon (using 200 mg activated carbon per liter instead of 100 mg raises the recovery of 100 $\mu g\ l^{-1}$ selenite to 82 % after a 15 minutes contact time).
The binding of the selenium on the activated carbon is quite strong: when a Nuclepore filter, loaded with activated carbon and 1 μg Se is rinsed with 1 l of bidistilled water, no losses of Se can be observed. The capacity of adsorption of activated carbon in this batch mode was compared with that of an activated carbon column as used by Massée, van der Sloot and Das (1977) for 1 l of a 10 $\mu g\ l^{-1}$ selenite solution. No difference was noted.

Influence of Various Salts.

For the recommended analysis conditions, it was found that neither at the 0.5 nor at the 5 µg l^{-1} selenite level, the recovery changed drastically in a solution 3.5 % or even 15 % NaCl or when 10, 100 or 1000 ppm of Fe^{3+} was present.
Also the presence of 50 ppm to 5000 ppm of Mg $SO_4.7H_2O$, $Na_2CO_3.10H_2O$, NH_4Cl and $CaCl_2.2H_2O$ did not influence the recovery of 0.25 µg/l selenite to a significant extent.

Influence of Humic Material.

Tracer experiments involving solutions (pH 2) with 0.3, 1.2 and 10.2 µg l^{-1} of selenite to which 0.4 to 6 ppm of fulvic acid had been added, showed that, for short contact times, the selenium recovery is not significantly influenced by the presence of fulvic acids. At an elevated level of 15 ppm of fulvic acid a 6 % recovery reduction was measured.

Risk of Selenium Losses by Adsorption on Container Walls.

Tracer experiments showed that losses of selenite on pyrex and polyethylene containers were negligible, even at the µg l^{-1} level and after prolonged storage up to 20 days.
Selenite solutions (0.3 ppb to 10 ppb, pH 2) containing 0.4 ppm of humic substances were stored for 18 h in polyethylene bottles. The loss, due to adsorption, after this time of contact and for the whole concentration range was 0.3 %. After a contact time of 20 days, the loss was raised to 9 % (0.3 ppb selenite solution) and 2 % (10 ppb selenite solution).
Elemental selenium, however, is lost rapidly on both materials. The addition of activated carbon does prevent these losses, however. Therefore in the analysis procedure the activated carbon should be added immediately after the reduction step with ascorbic acid.

Reproducibility.

A solution with 0.5 µg l^{-1} of selenite was analysed in eigthfold by the proposed procedure. An overall coefficient of variation of 8 % was noted.

Detection Limit.

From the selenium blank and background scatter contributions, the detection limit for the XRF procedure is calculated to be at the 0.05 µg l^{-1} level. This is quite satisfactory for most environmental applications.

CONCLUSION

The proposed procedure for selenite determinations in 1 l environmental water samples involves the reduction of selenite during 15 minutes after the addition of 3 g l-ascorbic acid, the adsorption of the resulting elemental selenium onto 100 mg activated carbon, the filtration of this carbon on a

Nuclepore membrane in a 10 cm^2 active area filtration unit, and the measurement of the characteristic Se-K_α peak on the resulting thin target by energy-dispersive X-ray fluorescence.
This procedure provides for a constant selenite recovery around 90 % for the normal environmental range between 0.05 and 3 $\mu g\ l^{-1}$ of selenite.
The recovery is not critically dependent on the salt content or humic material concentration. At the 0.5 $\mu g\ l^{-1}$ level a 8 % precision is achieved. The detection limit is around 0.05 $\mu g\ l^{-1}$ of selenite.

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Comparison of Methods of Trace Element Enrichment for XRF Determination

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ABSTRACT

In recent years, considerable progress has been made in the field of metal preconcentration from environmental water samples. The methods most widely used include ion-exchange, precipitation (and coprecipitation), adsorption and chelation with immobilized and non-immobilized reagents. As the mechanisms underlying the different methods are different, it is expected that these methods perform differently with respect to the enrichment factor, sensitivity and recovery of trace metal ions. In this investigation, the preconcentration of 10 cations (Cr, Mn, Fe, Co, Cu, Zn, Ag, As, Hg and Pb) was studied. These metals were chosen on the basis of environmental impact as well as on the basis of chemical and x-ray spectroscopy problems. The methods of preconcentration include (1) cation exchange resin-loaded filter paper, (2) precipitation with sodium-diethyldithiocarbamate, (3) precipitation with ammonium pyrrolidinedithiocarbamate, (4) chelation with oxine and subsequent absorption on activated carbon and (5) chelation with diethyldithiocarbamate immobilized on controlled pore glass. All of the methods were carried out exactly as published to get a realistic picture of their suitability for a routine laboratory method. Among the factors compared are the detection limits, sensitivity, precision, linearity and dependence of the recovery on the concentration of metal ions. This is a preliminary report of some of the results.

KEYWORDS

Trace Analysis, Preconcentration, Enrichment, X-ray Spectrometry, Elemental Determination.

INTRODUCTION

X-ray fluorescence spectrometry for water analysis at environmentally relevant levels of trace ions frequently requires a preconcentration step to insure adequate detection limits (Leyden, 1977). The good spectral resolution of XRF instruments have lead to the development of unselective preconcentration pro-

cedures, most of which yield thin samples for better sensitivity and reduced matrix effects. However, literature data on preconcentration are of limited use in the selection of the most appropriate technique for a number of reasons. Experimental conditions vary over a wide range, interference studies are rather limited, two or more studies invariably come up with different optimal parameters, very few real samples are run, detection limits are frequently extrapolated and sensitivities are obtained on different instruments. Many of these drawbacks are eliminated if one laboratory attempts the task to evaluate different methods.

The methods studied in this research are all taken from literature and are applied as published with the exception of very obvious modification where necessary. It was decided as an initial study to compare the ion exchange resin-loaded filter papers (Campbell, Spano and Green, 1966; Carlton and Russ, 1976), a column method utilizing dithiocarbamate immobilized on controlled pore glass (Leyden and Luttrell, 1975; Leyden and co-workers, 1976), precipitation with sodium diethyldithiocarbamate (Watanabe, Berman and Russell, 1972) and precipitation with ammonium pyrrolidinedithiocarbamate (Morris, 1968), as well as the adsorption of oxinates on activated carbon (Vanderborght, Verbeeck and Van Grieken, 1977; Vanderborght and Van Grieken, 1977a). The trace ions studied included Cr^{+++}, Mn^{++}, Fe^{+++}, Co^{++}, Cu^{++}, Zn^{++}, Hg^{++}, Pb^{++}, As^{+++} and Ag^{+} and were chosen to represent a reasonable compromise between ions of environmental importance and ions that are expected to cause problems in the preconcentration step or x-ray spectrometry. The particular objective of this work was to obtain basic figures-of-merit (e.g. detection limit, linear dynamic range, sensitivity, precision) under ideal one-element-at-a-time conditions and to obtain sufficient interference data to explain discrepancies in the analytical results on environmental water samples. Practical experience is presented to show to what extent energy dispersive and/or wavelength dispersive x-ray fluorescence spectrometry can be utilized for the determination of dissolved ions in natural waters.

EXPERIMENTAL

Apparatus. A Philips model PW-1410 x-ray fluorescence spectrometer was used for all wavelength dispersive measurements. In all cases a LiF-200 analyzing crystal was used with gas flow and/or scintillation detector. A Mo, W or Cr x-ray tube was operated at suitable kV and mA settings. The counting times varied, but did not exceed 100 s for the lowest concentrations and the blank. All spectrometer operations were under control of a Nova-1220 computer. The energy dispersive x-ray fluorescence analyses were performed on a Spectrace NS-440 spectrometer from Nuclear Semiconductor (United Scientific Corporation) equipped with an automatic 20 position sample changer and a filter wheel with 5 source filters and interfaced to a NS-880 analyzer from Tracor Northern. A low wattage Ag x-ray tube (Watkins-Johnson) was operated in pulsed mode unless otherwise stated. The voltage was set to 30 kV for all elements except Hg^{++} for which it was set to 40 kV. Current was adjusted depending on the sample preparation technique to give a maximal system deadtime of less than 50%.

A 0.25 mm Ag filter was used to lower the background caused by backscattered tube radiation. The process was controlled by a PDP 11/05 computer using the special purpose language FLEXTRAN supplied by the vendor. The counting time was 1000 s for the blank and 1 μg samples. All the other data were collected in 100-200 s counting time.

Reagents. The stock solutions were prepared from reagent grade chemicals and $CrCl_3 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $FeSO_4 \cdot 7H_2O$, $CoCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$ $HgCl_2$, $CdCl_2 \cdot 2\frac{1}{2}H_2O$, As_2O_3, $Pb(NO_3)_2$ and $AgNo_3$ were used. The $Ca(NO_3)_2$ and NaCl was supplied by J.T. Baker and Mallinckrodt in reagent grade quality. Sodium diethyldithiocarbamate (NaDDTC) and ammonium pyrrolidinedithiocarbamate (APDC) were solutions prepared by dissolution of the reagents in deionized water. (0.1 and 1.0 wt/wt% respectively) Before use, these solutions were filtered through a glass-fiber filter and made up fresh daily.

The activated carbon was purified according to a previously published procedure (Vanderborght and Van Grieken, 1977b). The oxine (8-hydroxyquinoline, Baker) was dissolved in acetone (~500 mg/mL) and kept for not more than a week at 4° C.

The ion exchange resin-loaded filter papers (SA-2 from Reeve-Angel) were washed with three-40 ml portions of saturated NaCl solution and afterwards with 5 ml deionized water. This converted the ion-exchanger to the Na^+-form and removed at the same time major portions of the Ca and Fe contamination of the commercially available product.

Glass beads (Electro-Nucleonics, CPG-10, 200-400 mesh) were used as substrate for the immobilization of N-β-aminoethyl-γ-aminopropyltrimethoxysilane (Dow-Corning Z-6020).

All other reagents were of reagent grade.

Preconcentration Procedures. The wetted ion exchange resin-loaded filter paper (25 mm in diameter) were placed in a Millipore filtration apparatus. Up to six of these filtration apparatii could be used in parallel, the vacuum generated by a Cenco HyVac-2 vacuum pump and connected to the flasks via a liquid N_2 filled cold-trap. The water sample was brought to pH 3 ± 0.05 and filtered through the ion exchange disk at a filtration rate of 40 ± 10 mL/min. The filtration step was repeated five or seven times after it was established that once or twice was insufficient. The disks were dried and sandwiched between two pieces of mylar held on a Chemplex #1430 sample cup.

The diethyldithiocarbamates were formed by adding 5 ml of the 0.1% solution of the reagent to a sample that had been adjusted to pH 4 with 2 ml of 0.1 M KHP buffer. The solution was filtered through a Gelman 0.45 micron filter after standing for about 15 min. The APDC precipitates were prepared similarly, but they were stirred for 2-3 minutes and aged for not less than 20 min (Morris, 1968).

The formation of the oxinates was accomplished by adjusting the pH value to 8 ± 0.1 with NH_4Cl/NH_4OH buffer and adding a predetermined amount of oxine solution according to the formula given by Van Grieken and coworkers (Vanderborght, Verbeeck and Van Grieken, 1977; Vanderborght and Van Grieken, 1977a). This formula takes into account the amount of oxine needed to complex Ca^{++}, Mg^{++} and the trace ions plus an excess of 5 ppm of reagent. 100 mg activated carbon was added to the sample. The solution container was then rotated for an hour to adsorb the oxinates on the carbon surface. Afterwards the carbon was filtered onto a Gelman filter and mounted in moist state on a Chemplex cup. The lower support of the carbon carrying filter had to be a mylar film with small holes punched as drying of the sample was done in vacuum after mounting. This procedure was adopted because of the difficulty in

handling the dry activated carbon.

The controlled pore glass (CPG) reacted with N-β-aminoethyl-γ-aminopropyl-trimethoxysilane was prepared according to a previously published procedure (Leyden, 1977; Leyden and Luttrell, 1975) and the capacity was determined by batch equilibration with Cu^{++} to be 0.5 mmol/g. From this the bisdithiocarbamate was formed by reacting 20 mL CS_2, 25 mL 0.25 M NaOH, 25 mL 2-propanol and 20 g CPG for 20 min at room temperature. The slightly yellow product is filtered off and washed with 50 ml portions of 2-propanol and deionized water until the aqueous filtrate is clear. After air drying it is refrigerated and kept for 10 days at the longest. 200 mg of these glass beads are filled in a 50 mm long PTFE tubing and connected water tight with PTFE screws (Fluoroware, Inc.). The ends are covered with Bio-Rad frits. The preconcentration is achieved by pushing the water sample adjusted to pH 7 with NH_4Cl/NH_4OH buffer through the column preconditioned by wetting with the same buffer. The pressure was supplied by compressed nitrogen and 25 psi were sufficient to achieve flow rates of approximately 30-40 ml/min. The vessels were rinsed with deionized water and this washing solution was pumped through the column as well. The columns can be opened immediately and the glass beads are dried in an oven at 70° C. Afterwards they are homogenized and held between two mylar foils. This preparation procedure is advantageous because of lower backscatter than the previously employed pelletization and does not lead to greater random errors than the preconcentration step itself (Leyden and Luttrell, 1975).

Calibration Curves. For all five methods, single element calibration curves were established with 16-100 mL samples, 5 blanks, 5 at 1 μg and two each at 20, 50 and 100 μg, assuming a constant relative standard deviation and establishing the linear range of each method (Hubeaux and Vos, 1970).

RESULTS AND DISCUSSION

For routine analysis of environmental water samples a simple and rugged method of preconcentration is of importance. This means that reliable multi-element preconcentration has to be achieved for a wide variety of environmental water types at the ppb-level. Initial studies were conducted for the ion exchange method and the two precipitation methods to establish the optimal working pH. For the ion-exchange method a pH-value of 3 was found to be a good compromise for the trace ions selected for investigation and a tolerance of ±0.2 pH-units seemed to be acceptable (Leyden, Ungerman and Nonidez, 1977). The dithiocarbamates gave the best overall recovery at a pH of 4±0.2. The other two methods have been investigated in this respect in previous papers (Leyden and Luttrell, 1975; Leyden and co-workers, 1976; Vanderborght and Van Grieken, 1977a) and a pH-value of 7±0.2 and 8±0.2 have been found to give good recovery for the immobilized dithiocarbamate and the oxinates adsorbed on activated carbon, respectively.

Recovery data have been reported in literature for all five methods and for the majority of elements the recovery was found satisfactory under the conditions of the analyte ion and the abundance of concomitant ions. In the absence of concomitant ions, the relative recovery at 200 ppb was taken as reference and ratioed with the relative recovery at 10 ppb. Table 1 gives selected data for preconcentration from 100 mL solution. Dashes are shown where either no recovery is achieved or the 10 ppb signal was below the detection limit. The errors given are composed mainly of the error of the back-

ground estimation and the error in estimating the signal at 10 ppb. The recovery of the ion-exchange paper seems to drop towards higher concentrations, while the precipitation methods work better at higher concentrations. Only the combined chelation and adsorption as done in the activated charcoal procedure appears to be independent of concentration in the range studied. No data are given for the immobilized dithiocarbamates as the thicker samples yield lower sensitivities and 10 ppb of ions from 10 mL (1 μg) gives no detectable signal.

Table 1: Recovery at 10 ppb Relative to Recovery at 200 ppb (%)

Element	Cr	Mn	Fe	Co	Cu	Zn	Hg	As	Pb	Ag
SA-2	--	121±9	--	132±15	98±34	--	--	--	91±19	--
NaDDTC	--	--	51±12	67±16	115±22	30±28	61±12	--	21±9	--
APDC	--	--	72±41	84±20	84±20	--	46±12	81±15	--	--
Oxine/AC	--	94±27	--	--	--	--	123±22	--	110±18	--
CPG	--	--	--	--	--	--	--	--	--	--

Calibration curves were constructed with 16 samples. The slopes of these calibration curves were used to obtain the sensitivity (cps/μg) for each element by each method. The results are summarized in Fig. 1.

The spacing of the concentration range (0-100 μg) was chosen to give reliable estimates of the blank and 1 μg level signal and to establish the linear range. The six values between 20 and 100 μg could be used to estimate the precision of the methods. The upper limits of the linear range and the correlation coefficients of the calibration function are given in Table 2. Of the five methods, the activated carbon/oxinate procedure as used seems to be the one with the most limited capacity.

The elements that give consistently low correlation coefficients are mercury, zinc and iron, presumably because of the enhanced risk of laboratory contamination. Another feature of the chosen subdivision of the calibration range was the fact that fairly reliable estimates of the blank and its standard deviation and the precision at low concentrations could be obtained. This and the fact that the calibration points are centered at fairly low concentrations made a good estimate of the decision and detection limits possible. The decision limits have been calculated according to Currie's suggestion (Currie, 1968) by taking a multiple of the blank standard deviation and are given also in Table 2. The values lie generally between 0.1 and 10 μg. The only case where they are considerably higher is for the chromium determination with the the wavelength-dispersive x-ray system. This appears to be a problem of excitation.

CONCLUSION

Of the several preconcentration methods investigated, most combinations of ion and method gave good recovery from pure, standard solutions. However, all show some degree of interference. Examples are the displacement of monovalent ions by Ca^{+2} on ion exchange resin-loaded filter paper. Other types of problems are slow reaction kinetics such as Cr^{+3} with APDC and low sensitivity because of matrix dilution. The results presented here are only

Table 2: Upper Limits of Linear Range (μg), Correlation Coefficients and Decision Limits (μg)

Elements	Cr	Mn	Fe	Co	Cu	Zn	Hg	As	Pb	Ag
SA-2	100[1]	100	100	100	50	100	50	--	100	100
	0.997[2]	0.9993	0.9993	0.993	0.9997	0.993	0.96	--	0.9991	0.999
	2/10[3]	0.2/07	1/2	0.2/--	0/0.5	3/--	9/0.2	--	0.2/--	--/3
NaDDTC	--	--	100	100	100	100	100	100	100	100
	--	--	0.998	0.999	0.9999	0.993	0.995	0.85	0.992	0.9991
	--	--	0.2/0.4	0.1/0.2	0.1/2	0.4/--	0.2/--	--	0.1/2	--/2
APDC	--	--	100	100	100	100	50	100	100	100
	--	--	0.998	0.999	0.998	0.995	0.998	0.994	0.998	0.997
	--	--	0.7/0.6	0.3/0.2	0.2/1	0.6/--	0.1/0.2	0.1/--	0.2/0.4	--/3
Oxine/AC	50	100	100	50	50	100	50	--	100	100
	0.996	0.998	0.994	0.994	0.9995	0.993	0.98	--	0.997	0.9992
	2/16	0.4/0.2	1/1	0.4/2	0.4/2	1/--	0.3/0.1	--	0.1/0.2	--/2
CPG	100	100	100	100	100	100	100	--	100	100
	0.96	0.998	0.98	0.97	0.99	0.98	0.98	--	0.996	0.89
	8/70	0.9/1/0	-/-	-/-	0.8/8	-/-	0.8/1	-/-	0.4/--	-/-

[1]Upper Limit of Linear Range [2]Correlation Coefficient [3]Decision Limits (EDS/WDXRF)

SA-2: Ion-Exchange Paper
NaDDTC: Sodiumdiethyldithiocarbamate
APDC: Ammonium Pyrrolidinedithiocarbamate
Oxine/AC: Activated Carbon and Oxinates
CPG: Controlled Pore Glass

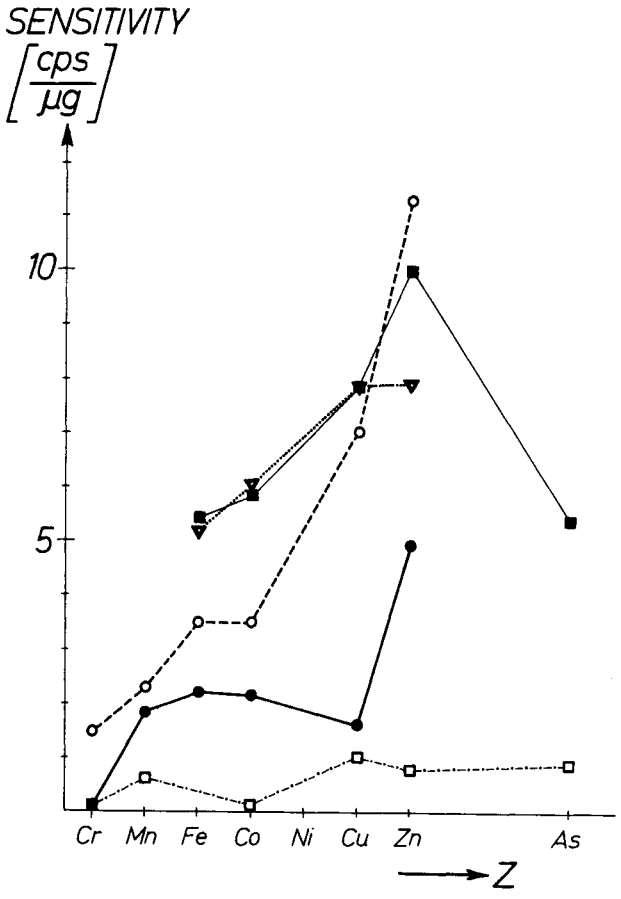

Fig. 1. Sensitivities for energy-dispersive x-ray spectrometry, Ag-tube, 30 kV, 0.025 mm Ag filter
○ Ion Exchange resin-loaded paper
■ NaDDTC precipitation
▽ APDC precipitation
● Activated carbon/oxinates
□ Controlled pore glass

indications of factors to be considered when preconcentration methods are used and show the need for further detailed study.

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Use of Electron Microscope X-ray Analysis in the Determination of Detoxication Mechanisms for Heavy Metals in Shellfish

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ABSTRACT

The application of electron microscope x-ray analysis to the investigation of the subcellular localisation of heavy metals in polluted marine shellfish has been discussed. A hitherto unrecognised detoxication mechanism has been demonstrated by this technique.

KEY WORDS: cadmium, detoxication, electron microscopy, heavy metals, lead, mussel, oyster, pollution, shellfish, x-ray microanalysis, zinc.

INTRODUCTION

The hazards of heavy metal pollution in the aquatic environment as a result of our increasing industrial activity has given us an abundance of chemical data for metal concentrations in key species. This monitoring of changes is essential. There has also been strong emphasis on dynamics of metal uptake in many of these organisms. However, our understanding of the mechanisms of uptake, storage and excretion of metals is sparse. In our laboratory we are concerned with the elucidation of these mechanisms in the hope that predictive models can be developed as an aid to structuring realistic pollution control programs. Our understanding of the chemical mechanisms of ion and metal transport must take into account the exceedingly complicated fine structure of cells and tissues revealed by electron microscopy. By use of electron probe x-ray microanalysis, elements within the substructures of cells and tissues

can be identified and quantitated and we can therefore make attempts to correlate structure and function.

ELECTRON PROBE X-RAY MICROANALYSIS IN BIOLOGY

Electron probe x-ray microanalysis was developed by Castaing in the late 1940's and it has been applied to the analysis of non-biological materials for many years, but the limited instrumental sensitivity and lack of suitable preparatory procedures have delayed its use in biology where we require reliable quantitation of elemental concentrations in microareas, with a simultaneous observation of fine structure. At the current stage of development, the technique can be usefully applied but cannot as yet satisfy all our requirements. The application of the technique to biology has been extensively reviewed (Echlin & Galle, 1975; Gupta, Hall & Moreton, 1977; Hall, Echlin & Kaufmann, 1974). Ideally elemental concentration data requires the preservation of both fine structure and diffusible soluble materials in their own in vivo state and localisation, without being altered during specimen preparation or analysis. This is most closely approached when the tissue is not chemically fixed but is deep-frozen and analysed in the deep-frozen hydrated state (Gupta, 1976; Moreton and others, 1974). However, in this condition, fine structural information is limited and there are many situations where other specimen preparation techniques provide useful information. In this paper we shall place emphasis on these less exacting qualitative methods in our heavy metal pollution studies of marine bivalve shellfish.

INSTRUMENTATION

The specimen images may be formed by transmission (TEM) or scanning electron microscopy (SEM). In the former the electron beam is stationary and image formation is by lenses; whilst in SEM, a finely focused beam scans the specimen and the image is formed on a synchronously scanned cathode ray tube, the image being obtained by detection of absorbed, transmitted, back-scattered or secondary electrons (or emitted x-rays). For TEM, the specimen is generally a thin section, whilst bulk specimens or thicker sections can also be used for SEM. Two different kinds of x-ray spectrometers can be used, energy selective systems based on solid-state (lithium-drifted silicon) detectors which simultaneously detect many elements but cannot detect elements of atomic number less than 11 and wavelength selective systems, based on diffracting crystals which have the advantage of high peak/background resolution and usefulness for light elements such as Na, but can only measure one element at a time.

For theoretical and technical reasons the system which is most useful for biological applications should be capable of analytical spatial resolution better than 0.5 μm (therefore using thin sections and accelerating voltages of 60KV or higher) and have scanning facilities, so that image contrast can

be enhanced electronically (since the intrinsic contrast in unstained and especially hydrated, sections is very low). The transmission scanning (STEM) mode and the facility for observation of frozen hydrated sections i.e. using a cold stage maintained at below -150°C, is probably most useful.

In our laboratory we use a JEOL 100CX electron microscope equipped with a scanning unit and a Kevex energy dispersive x-ray spectrometer. At present it is not fitted with a cold-stage and cryo sections have to be freeze-dried or plasma incinerated at ultra low temperature.

PREPARATION OF TISSUES

The ultimate aim is to preserve the ultrastructure as well as possible while maintaining a faithful elemental composition. It would be ideal to examine tissues directly. However, soft tissues are not sufficiently hard to permit the cutting of sections less than 1 µm thick, and the cellular contents of cut cells would "run out". Moreover, wet specimens cannot be put in the instrument as it operates in a very high vacuum. There are two approaches to the problem. The best is to rapidly freeze-clamp the cells within a fraction of a second at a temperature below -64°C, the point of ice crystal formation (usually about -200°C is used), then to section and examine it whilst still frozen. The protocol for this procedure has been discussed recently by Gupta, Hall & Moreton (1977). The other approach is to replace the water in the specimen with plastic and cut sections of this material. Soluble electrolytes are unlikely to remain in their in vivo situation when tissues are immersed in organic solvents. Elements may also be present in both the bound form and as free ions, and therefore they will be affected differently during specimen preparation. Radioactively labelled tissues or atomic absorption spectrometry can be made use of to determine elemental losses during specimen preparation (George and co-workers, 1976; Mehard & Volcani, 1975; Morgan, Davies & Erasmus, 1975).

In some cases conventionally fixed, dehydrated and embedded material may be used for microanalysis but a great deal of care must be exercised in the interpretation of the results obtained. The distribution of particulate metals is usually unaffected by chemical treatments and successful studies have been carried out with calcified fish tissues (Cowey and co-workers 1977) and particulate ferric hydroxide in *Mytilus* (George, Pirie & Coombs, 1976).

Many cytochemical techniques for localisation of molecular groups are suitable for x-ray microanalysis. These methods have been reviewed comprehensively (Chandler, 1975; Lauchli, 1975; Pearse, 1972). The most commonly used methods for the subcellular precipitation of cations are the use of potassium pyroantimonate, which has been used successfully for calcium and zinc (Chandler, 1975; Chandler, Timms & Morton, 1977) and sulphide, which has been used for cadmium, copper and zinc (George and co-workers, 1976; George and co-workers, 1978). It must be remembered that histochemical reactions depend upon diffusion of the reactants into the tissue, obviously freely soluble elements can diffuse similar distances before reaction and precipitation occurs, therefore these methods can only be usefully employed where the elements are bound or compartmentalised, so that their diffusion is much reduced.

Examples of the application of electron probe x-ray microanalysis in our studies of heavy metal metabolism in estuarine bivalve shellfish are illustrated in the following paragraphs.

STUDIES WITH METAL EXPOSED MUSSELS

Biochemical experiments have been carried out on the common mussel, *Mytilus edulis*, exposed to sublethal levels of iron, zinc, lead or cadmium and the kinetics of uptake and excretion have been measured (Coombs, 1977; George & Coombs, 1977; George, Pirie & Coombs, 1976; George and co-workers, 1978). These metal exposed animals were also used for electron probe x-ray microanalysis. Iron is normally present in sea water as particulate ferric hydroxide and by a combination of ^{59}Fe radiolabelling and electron microscope x-ray microanalysis we showed that it was taken up by a process of endocytosis i.e. engulfment of the metal by the epithelial cell membrane, which then pinches off to form membrane-limited vesicles inside the cell. The vesicle then passes to the basal end of the cell where by the reverse process of exocytosis it is expelled into the blood. Here the circulating amoebocytes reabsorb the metal by endocytosis and transport the metal to the kidney and other tissues for storage and eventual excretion (George, Pirie & Coombs, 1976). The kidney of *Mytilus* contains very high concentrations of iron and heavy metals, after exposure to 100 µg/l Fe, Cd, Pb or Zn (in separate experiments) for 25 days, the concentrations of the respective metals were 10.2, 1.1, 0.7 and 1.2 mg/g wet tissue. Samples of the kidney were processed for electron microscopy by conventional procedures -- glutaraldehyde/formaldehyde fixation (osmium tetroxide is avoided because its mass increases the Bremmstrahlung), alcohol dehydration and resin embedding. Metal losses were monitored by radioactivity (^{59}Fe, ^{109}Cd. and ^{65}Zn) or atomic absorption spectrometry (Pb). Retention varied between elements -- whereas greater than 80% of the iron and zinc were retained, only 45% of the cadmium was retained. Examination of sections in the analytical electron microscope showed that the iron was confined within membrane-limited vesicles (Fig. 1A), whilst there was evidence of redistribution of zinc and lead (precipitation along cell interfaces). Cadmium was not detectable, indicating a uniform distribution (George and co-workers, 1976). Use of hydrogen sulphide saturated fixative increased the retention of cadmium to 60% and removed the distribution artifacts previously observed with lead and zinc. As with iron, both lead and zinc were found to be localised in membrane limited vesicles (Figs. 1B,C and spectra Figs. 1D,E). The x-ray spectral data showed that the elemental composition of the vesicles is variable but they nearly always contain calcium iron, zinc and lead. After specific metal exposure the content of that metal increases and becomes the predominant species. Traces of calcium were detected in mitochondria and occasional traces of lead were found in nuclei. Despite increased retention of cadmium after sulphide treatment the metal could not be detected by microprobe analysis. This is probably to be expected since a major portion of the cadmium is complexed with a specialised protein, metallothionein, (George and co-workers, 1979), and this protein in vertebrates is predominantly soluble after physical fractionation techniques. However, we attempted to localise the metal by use of cryo procedures - unfixed tissue was rapidly frozen in liquid nitrogen sluch (-216°C), sectioned at -110°C in a cryomicrotome, freeze dried at -80°C and then analysed (George & Pirie, 1979). Structural details were very limited, cellular outlines, electron dense granules, nuclear and mitochondrial profiles could be observed only with the image enhancement capabilities of the STEM mode. The x-ray analysis of the granules (Fig.1F)

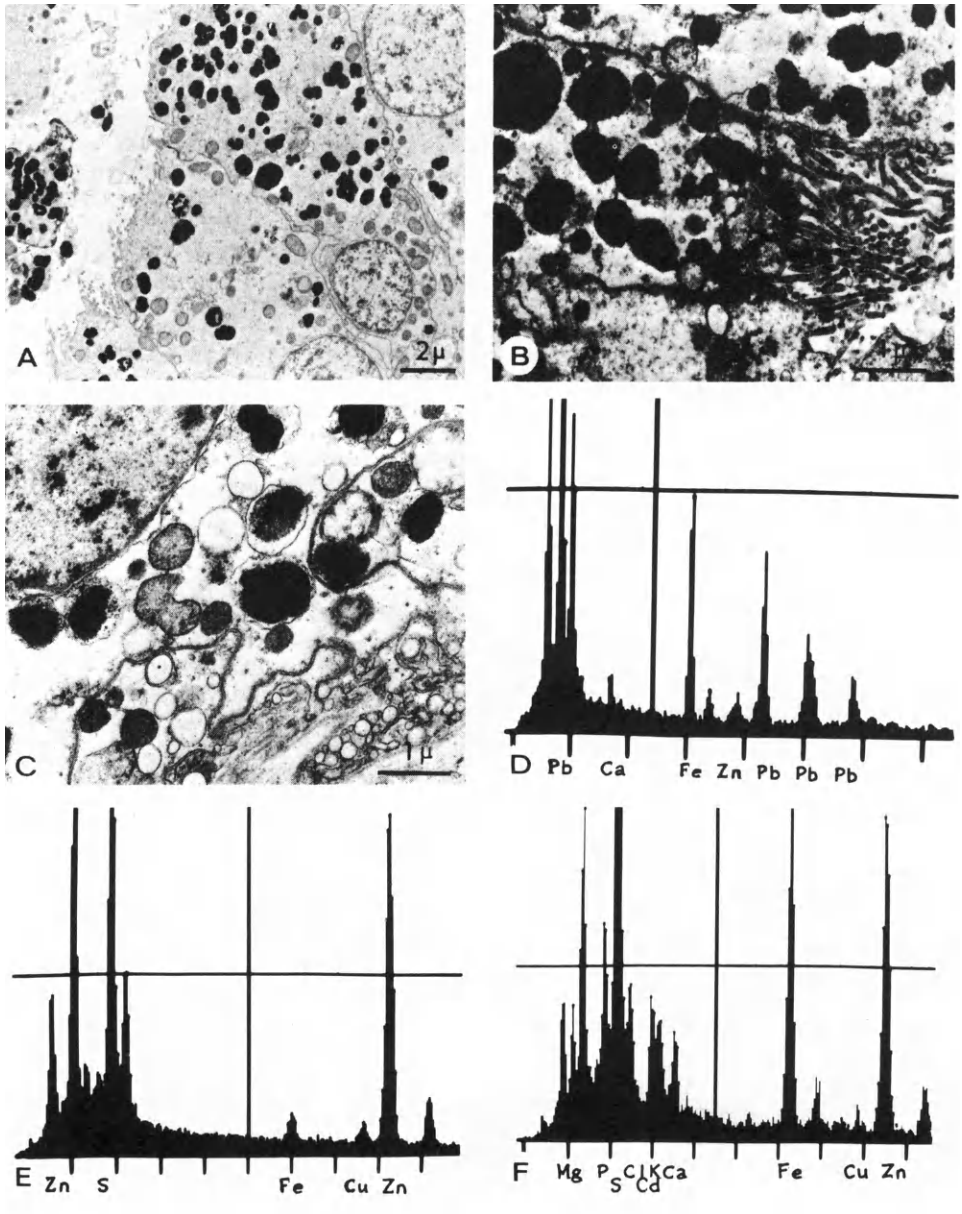

Fig. 1. Kidney of *Mytilus*. (A) Section of tubule from Fe-exposed kidney showing Fe-containing granules. (B) Showing apical region of cell in Pb-exposed animal. (C) Basal region of cell from Zn-exposed animal showing membrane-limited vesicles containing metal. X-ray spectra of granules from resin sections of H_2S fixed Pb and Zn-exposed animals (D & E respectively) and (F) cryo-sectioned Cd-exposed animal.

shows that the soluble elements, Mg, K, P, Cl etc., are retained by this method of preparation and that the granules contain Cd, Ca, Fe and Zn. Thus cadmium can also be found in membrane-limited granules in the kidneys of *Mytilus* after exposure to the metal. This clearly demonstrates that several experimental approaches should be made to this question of localisation when a metal is easily lost during specimen preparation. By careful biochemical fractionation, we have found that under conditions were 40% of the lysosomes have been broken, 80% of the total cadmium is in the soluble fraction. We have isolated a Cd-binding protein from *Mytilus* which has many of the characteristics of metallothionein, a low molecular weight sulphur rich protein (George and co-workers, 1979). As noted earlier, in mammals this protein is generally thought to be soluble and present in the cell cytoplasm. However, as we have found in *Mytilus*, there is always a variable proportion present in the particulate fractions. Now by use of cryo procedure for specimen preparation we have demonstrated the presence of high Cd and S concentrations in membrane-limited vesicles. We therefore conclude that the Cd-binding protein is localised in these structures. Since these vesicles also contain Ca, Fe, Pb and Zn, which are not all present in the purified Cd-binding protein, and display different retention characteristics during specimen preparation, then they must be found differently. Their complexation is currently being investigated.

STUDIES WITH NATURALLY POLLUTED OYSTERS

The subcellular localisation of copper and zinc in *Ostrea edulis* from estuaries polluted with mine tailings in Cornwall, England, containing elevated levels of these metals ("green-sick") was investigated (George and co-workers, 1978). The combined electron microscope and microprobe studies revealed structural compartmentalisation of the zinc and copper in separate, distinct granular amoebocytes (Fig. 2). The metals are immobilised as with *Mytilus* in membrane-limited vesicles within the amoebocytes, but each metal is associated with a different chemical compound, zinc with phosphorus and copper with sulphur. Unlike *Mytilus* these vesicles in *Ostrea* are not found

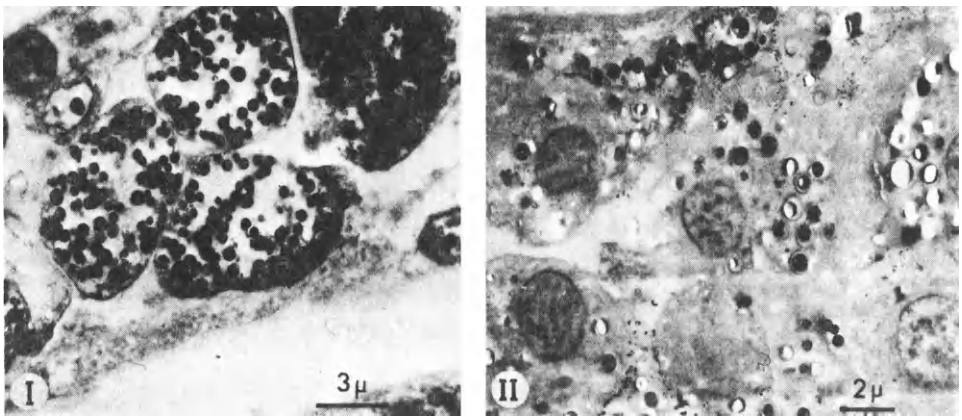

Fig. 2. Electronmicrographs of sectioned gill tissue from a "Green-sick" oyster obtained from Cornwall. Showing amoebocytes containing copper (cell type A) and zinc (cell type B). I. Fixed with H_2S saturated fixative and II. No H_2S, note redistribution of Zn.

in tissue cells but only in the amoebocytes, which can penetrate all of the tissues and are found mostly in the mantle and gills. Thus the tissue cells of the oyster have comparatively low metal concentrations and excess toxic metal is accumulated in these wandering amoebocytes.

CONCLUSIONS AND A CAUTIONARY NOTE

It therefore appears that these bivalve shellfish tolerate the extremely high concentrations of heavy metals within the tissues by isolating the potentially toxic metal within a membrane, thereby immobilising and detoxifying it. Stimulated by these findings we have found other examples of this mechanism in many animals from *Protozoa* to man (Coombs & George, 1978). Thus electron probe x-ray microanalysis has proved a useful technique in the determination of novel detoxication mechanisms.

Despite enormous advances in the use of the technique in biology it cannot yet answer many of our problems. The two most critical stumbling blocks are still specimen preparation and sensitivity. Although we can detect as little as 10^{-19} g of an element (e.g. a single ferritin molecule contains 10^{-19} g of iron or 5000 atoms) this would require a concentration of about 100 ppm in the area analysed. We are therefore limited at present to detecting localised concentrations.

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Studies of Trace Elements Movements in the Environment by X-ray Emission Spectroscopy

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ABSTRACT

Proton-induced X-ray emission (PIXE) spectroscopy allows simultaneous determination of the concentrations of all biologically essential trace elements and heavier toxic elements in variety of biological and environmental samples. In this report the applications of PIXE for trace element analysis of soils, waters and air particulates will be described. Elevated levels of trace elements in the environment are the result of human activities; very important is the use of fossil fuels and products. PIXE analysis allows the determination of "elemental fingerprint" which in turn helps the identification of pollution sources and pollutants movements. Measurement of trace elements levels in different biological tissues by PIXE reflects the exposure of living beings to elevated levels in the environment. Very useful are measurements of trace elements concentrations in blood, hair and some other tissues.

INTRODUCTION

Relationship between the environment and the living organisms is a very complex one. Of particular interest are the concentrations and movements of elements in nature. The bulk of living matter consists of eleven elements which have low atomic weight. In addition, some elements present in very small concentrations (trace elements) have been recognized as essential for life. The list of essential trace elements includes F, Si, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sc, Mo, Sn, and I. For an essential trace element there is a rather narrow range of adequacy of elemental concentration; many clinical and pathological disorders arise as a consequence of trace element deficiences and excesses (Underwood, 1971). In addition, a number of elements can be classified as toxic elements (Cd, Hg, Pb,...).

Proton-induced X-ray emission (PIXE) spectroscopy allows simultaneous determination of the concentrations of all biologically essential trace elements and heavier toxic elements in variety of biological and environmental samples (Valković and co-workers, 1974).

EXPERIMENTAL METHOD

In recent years the detection of characteristic X-rays for trace element analysis has increased significantly. The large cross sections for X-ray production allow the use of charged particles for the production of characteristic X-rays from elements present in trace amounts in target materials. We shall describe the method and system developed at the T. W. Bonner Nuclear Laboratories. Proton beams from the Rice University EN tandem Van de Graaff and CN 5.5 MeV Van de Graaff accelerators have been used in the measurements. The target chamber was constructed with optimum efficiency for the collection of produced X-rays. Care was taken to prevent the Si(Li) detector from seeing any high-Z materials which could produce fluorescence by beam scattering from the last beam slit or Compton scattered X-rays. In the chamber the target frames ride up and down on an aluminum target ladder which is milled off center so that the target faces are in the exact center of the chamber. For more details, see (Valković, 1977a).

In order to eliminate X-rays from elements below those of potassium and secondary electron induced bremsstrahlung below 3 MeV, a 0.1 cm polystyrene absorber was inserted between the target and the detector. A proton energy of 3 MeV was chosen to maximize sensitivity for the elements in the region of Fe to Zn (essential trace elements).

Special attention has to be paid to target preparations. Charged-particle induced X-ray emission has an advantage with respect to fluorescent techniques in that, because of the large cross sections, relatively thin targets can be used, and thus the matrix effects can be neglected. Thin biological and environmental targets are in general not self-supporting and must be prepared as deposits on some kind of backing. Different backings were investigated with the aim of finding one which would produce the least amount of background radiation and support the beam well. Any material to be deposited should be first reduced to a solution or a suspension of microscopic particles in an inert solvent. Pure water is the best solvent for most materials.

A very useful technique is the one involving the preparation of Al + formvar backings. By reinforcing aluminum foils with formvar one obtains backings which may be used in PIXE analysis. Formvar provides mechanical strength while the liquid or suspension is drying, and aluminum provides resistence on the beam of charged particles. Such a backing is free of any interference lines. Targets of water, blood serum and many solutions are very easily prepared by simply allowing a few drops to dry on such aluminum formvar backing. Absolute concentrations of trace elements in different targets were determined by doping the solution with yttrium and by measuring raw yields relative to yttrium which is not normally seen in biological and environmental samples in the ppm range. The relative efficiency of the system for different X-ray energies to yttrium was then measured by running yttrium-element pairs of atomic absorption standards.

RESULTS AND DISCUSSION

One of the most important characteristics of living beings is their ability to take up elements from a solution against the concentration gradient. The organisms concentrate all elements present in their environment.

Concentrations of elements which are present in fresh and sea waters in the

level of ppm or smaller have been of scientific interest for some time. Water pollution has received much attention since, over the years, water quality has deteriorated in many areas of the world. Increasing concern has been connected with the quantities of different metals, especially heavy ones like mercury and lead. Water targets are easily prepared by allowing a few drops to dry on an aluminum formvar backing. Such a simple target preparation allows the detection of elements present in the water with the concentrations $\geq$ 0.1 μg/ml. As an example, Fig. 1 shows the X-ray spectrum from the polluted seawater target; peaks associated with K, Ca, Fe, Cu, Br, and Sr are present (Y is dopant). Pollutants Fe and Cu are present in high enough concentration to be easily determined.

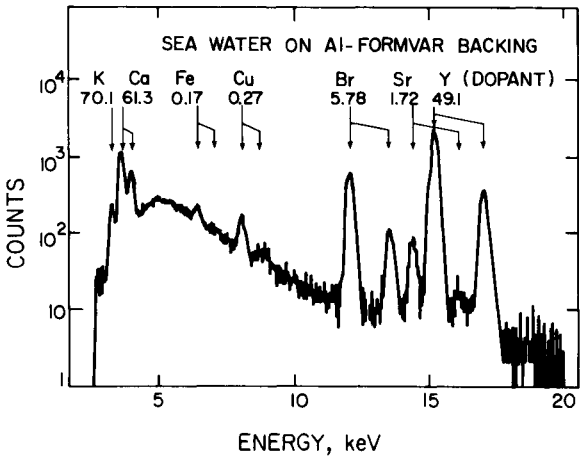

Fig. 1. X-ray spectrum of a seawater sample bombarded with 3 MeV protons. Target prepared by drying a few drops of seawater on Al-formvar backing.

For the analysis of dissolved trace metals present in water in smaller concentrations, a method involving formation of insoluble metal chelates coordination with dithiocarbamate (ammonium pyrolidine dithiocarbamage of diethyl dithiocarbamate), filtration through a membrane filter, and the analysis of the precipitate is often used. Simultaneous analysis of most of the transition elements is possible, but alkali and alkaline-earth metals are excluded. The following elements can be separated by dithiocarbamate: V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga. As, Mo, Rh, Pd, Ag, Cd, In, Sn, Sb, Pt, Au, Hg, Tl, Pb, and U (Pinta, 1962). The dithiocarbamate precipitation technique for target preparation offers several advantages over the procedures based upon evaporation, among them the uniformity of distribution of elements on the target, the simplicity which reduces both handling time and contamination hazard, detection limit of lo-3 ppm and lower, and high efficiency within a wide pH range. Figure 2 shows the X-ray spectrum from a seawater target prepared on the millipore filter from 0.1 liter of seawater. The target was bombarded with only 1-2 nA beam of 3 MeV protons.

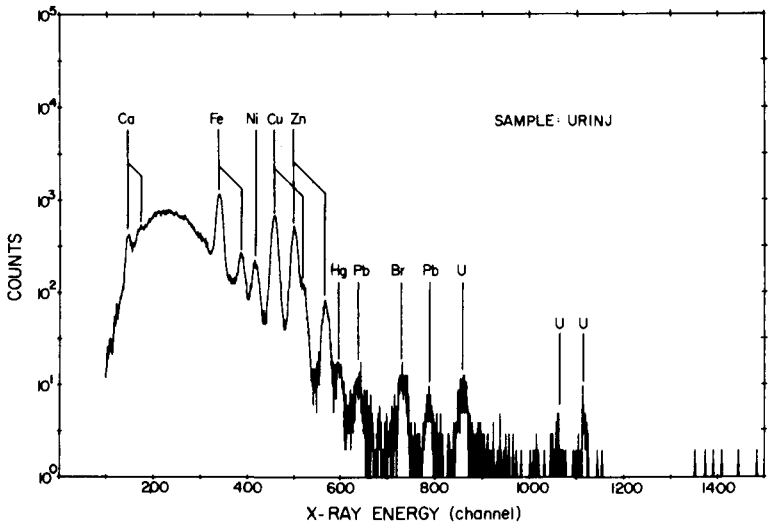

Fig. 2. X-ray spectrum from preconcentrated seawater target prepared on millipore filter paper from 0.1 liter of seawater by formation of insoluble metal chelates via coordination with ammonium pyrrolidine dithiocarbamate.

The biological experiments on the effects of pollutants are often done by introducing the controlled amounts of pollutant in the environment in which given species live. This is costumary procedure with metals and sealife. X-ray emission spectroscopy allows the determination of many metals in different parts of body simultaneously. As an example Fig. 3 shows the measured X-ray spectrum from fathead leaving in the Pb enriched environment. The sample was homogenized before the target preparation and yttrium was introduced as internal standard.

Animals living in Pb contaminated environment reflect to some extent this exposure by elevated levels in their tissues. It has been suggested that elemental analysis of honey can be used as an indicator of Pb pollution. We have significant amounts of Pb in honey originating from locations near the congested areas. Figure 4 shows the X-ray spectrum from one of the measured honey samples, with Pb concentration of 0.1 % in ash.

In the natural system (rock-soil-aqueous solutions-organisms) soils are an exceptionally important link (Valković, 1975). Trace element composition of soils can be easily determined by X-ray emission spectroscopy. When protons are used for excitation, only small amounts of material are needed for target preparation.

The use of fossil fuels is a widespread source of many pollutants. Pollution by crude oil and its products has attracted special attention (for details see Valković, 1978). Determinations of trace element concentration levels in crude oils and products have been described by many authors.

Charged particle induced X-ray spectroscopy has also been used in some laboratories. Some elements are present in oil in high enough concentrations that

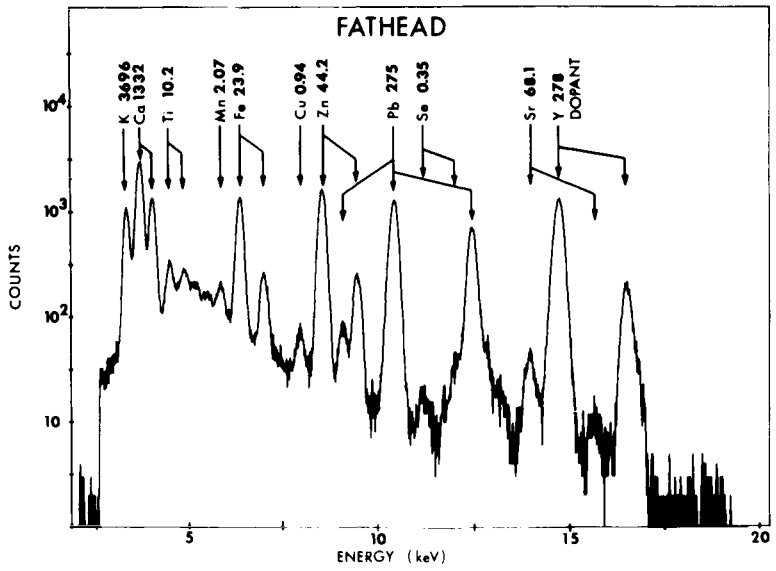

Fig. 3. X-ray spectrum from fathead living in Pb enriched environment. The target was prepared from homogenized tissue solution.

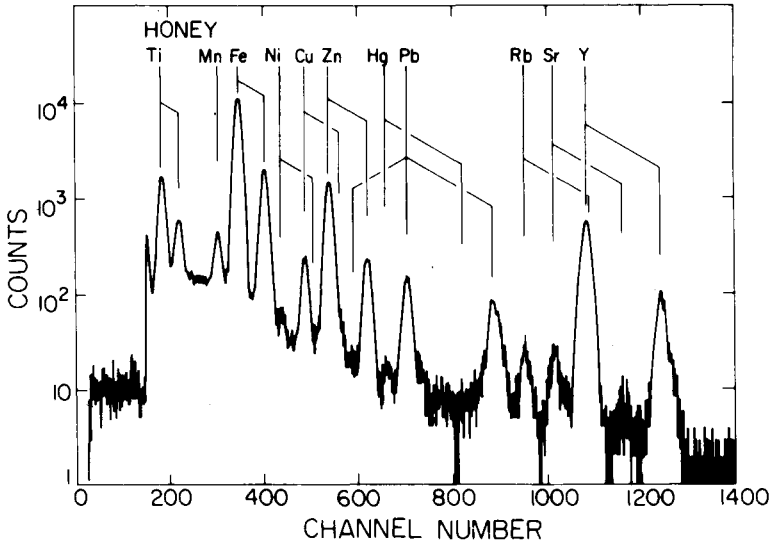

Fig. 4. X-ray spectrum resulting from honey sample; target was prepared by ashing.

they can be measured without preconcentration. For example, Fig. 5 shows an X-ray spectrum obtained by the bombardment of the target made by depositing a few drops of Venezuelan crude oil on filter paper. 3 MeV protons, I=10 nA, were used. The spectrum is dominated by bremsstrahlung radiation due to the target backing. However, peaks associated with S, V, and Ni can be easily identified. Concentration ratios, S/Ni=600 and V/Ni=20, are easily determined

with a measurement lasting only a few minutes. In order to be able to measure the other elements present in crude oil, dry ashing of oil was performed. Targets were then prepared by smearing ash on the backing.

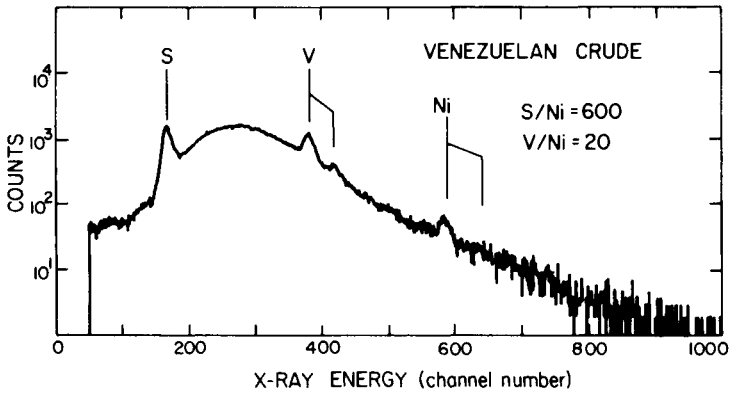

Fig. 5. X-ray energy spectrum obtained by the bombardment of target made by depositing a few drops of Venezuelan crude oil on filter paper.

Determination of wear metals in lubricating oil from aircraft and other motors has been studied by many investigators. Detection limits better than 1 ppm are reported for many metals. Figure 6 shows X-ray spectra obtained by the bombardment of new and used lubricating oil targets by 3 MeV protons. Targets were prepared by putting a few drops of oil on the filter paper. New motor oil reveals only peaks associated with zinc (which is a known additive to oil). The used motor oil from the author's car (1o ooo km) shows a variety of peaks; some of them can be associated with the additives of gasoline.

Concerns with air pollution and its effects on human health and disease have resulted in demands for fuels with lower and lower sulphur content. On-line systems for determination of sulphur based on the X-ray fluorescence technique have been described in literature. A system for continuous monitoring of lead levels in gasoline has also been developed. X-ray fluorescence analysis proved to be rapid and reasonably accurate to determine the concentration of about 2o elements in whole coal. Although major elements in coal - carbon, hydrogen, oxygen, and nitrogen - cannot be analyzed by X-ray fluorescence, most other elements at levels greater than a few ppm are readily determined. Trace elements determined by the X-ray fluorescence method are limited to those occurring in whole coals at a few ppm at least. The list of these elements can be a long one. Because of the speed and simplicity of the method, X-ray fluorescene is highly adaptable to large-scale surveys of coal resources.

Pollution by lead is often connected with the use of fossil fuels. Lead can be detected in both crude oil and coal using porton induced X-ray emission spectroscopy. Crude oils of different origin have different amounts of Pb. The same is valid for coals. It should be mentioned that the X-ray emission spectroscopy allows the determination of Pb within the "elemental fingerprint" of other elements. This is very useful in following the destiny of Pb in fossil fuels and products. The use of fossil fuels (and their products with

Pb-compound additives) is responsible for the majority of atmospheric load. X-ray emission spectroscopy is an accepted method for the determination of Pb content of air particulates. Figure 7 shows X-ray spectra of filter paper before and after air was pumped through it. Pb:Br ratio indicates the source of Pb to be automobile traffic.

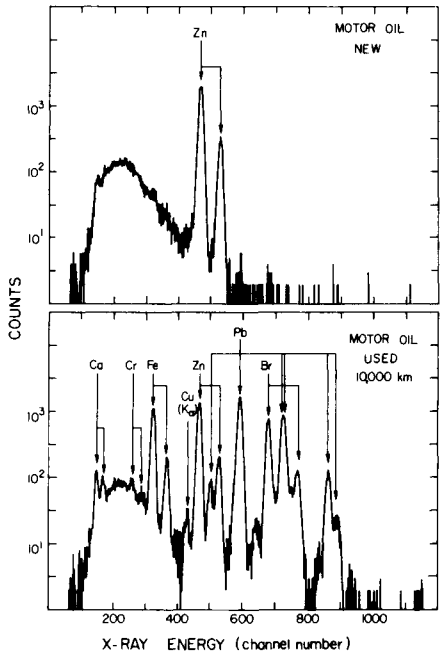

Fig. 6. X-ray energy spectra obtained by the bombardment of new motor oil and used lubricating oil (loooo km) targets by 3 MeV protons.

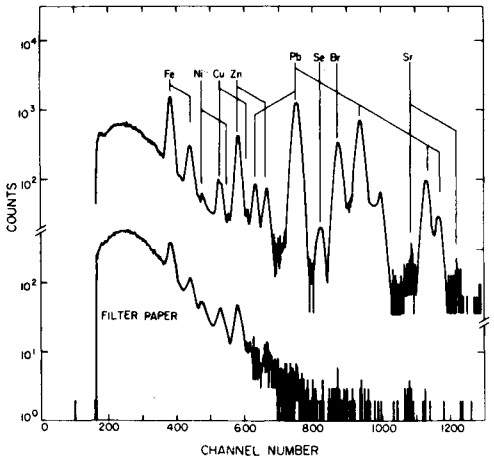

Fig. 7. X-ray spectra from the bombardment with protons of filter paper before and after air was pumped through it.

From the atmosphere Pb is transferred to soils, waters and plants. It is even incorporated into the structure of tree rings as a permanent record of atmosphere and soil loadings. We have investigated variations of Pb concentration across tree rings in an effort to establish variations in atmospheric load. This is still in progress.

Hair is a unique biologic material which reflects the biomedical and environmental history of the subject. Since it is convenient to handle and sample, and since it has relatively high concentrations of metals, trace-element analysis of human hair has been applied widely.

Because of its growth, hair reflects previous elemental concentrations in serum and body (history of previous biochemical and medical events in man), as well as previous environmental effects. Several measurements of trace element distributions along the hair length have been reported (Valković, 1977b). The investigators agree that the variations are characteristic of the subject and that Zn variations along hair are negligible. Assuming constant distribution of Zn along the hair, only the ratios of elemental concentrations to Zn concentration need to be measured to obtain elemental concentrations along hair. Such relative measurements can be easily performed on single hairs using proton-induced X-ray emission spectroscopy. It has been shown (Valković, Rendić, Phillips, 1975) that the elements whose concentrations increase monotonically along the hair can be identified as pollutants in

the area. For widespread pollutants (such as lead) even the medium value of a group of subjects can be used as a measure of the exposure. Actually, only elemental concentration ratios (relative to Zn) need to be measured.

CONCLUSIONS

In the past few years research interest in several disciplines has begun to focus on the complex problem of movements of elements in nature, and the relationship between the environment and living organisms. The development of trace element analysis by charged-particle induced X-ray emission spectroscopy as analytic technique suggests a nuclear accelerator laboratory as an important facility for the study of trace elements. Large cross sections for X-ray emission along with low background radiation allow the usage of thin sample targets in which matrix effects are negligible. Available computer facilities of a typical nuclear physics laboratory allow fast collection, processing and analysis of data.

ACKNOWLEDGEMENTS

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Proton Induced X-ray Emission Routine Analysis of Atmospheric Aerosols

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ABSTRACT

X-Ray emission induced by charged particles offers an attractive alternative to the X-ray tube or radioisotope source excited techniques. At the Department of Oceanography of the Florida State University, proton induced X-ray emission (PIXE) is used for routine quantitative analysis of 12 to 15 elements simultaneously in atmospheric aerosol samples.

Analysis is carried out by 5 MeV proton irradiation in a Van de Graaf accelerator, measurement of characteristic X-ray by Si(Li) detector and X-ray spectrum resolution by small computer.

Detection limits for multielement analysis are in the nanogram range and standard deviations are as low as 15 %. The methodology of the analytical procedure permits carrying out the analysis of more than 250 samples per 24-hours accelerator day, providing 5000 or more individual elemental determinations.

KEY-WORDS

Proton induced X-Ray emission, - Aerosols Analysis.

INTRODUCTION

Chemical composition of aerosol in the atmosphere falls in the range of the analysis wich requires higher sensibility. The nature of problem arised here is the necessity of large number of samplings (Vié le Sage and coworkers , 1978) and different sites possible. Both of these necessities drive the two departments, Oceanography and Physics, of Florida State University, Tallahassee, FLA., to develop the analyses of characteristic X-ray induced by proton beam as routine analysis method of aerosol samples.

This method is fast, broad range, absolute, subject to automation and well suited for samples having areas of a few square millimeters and thicknesses of 1mg/cm^2 (or approximately 10 µm). Samples of uniform density are most convenient, however, non uniform samples may be analysed by using a homogeneous proton beam.

AIR PARTICULATE SAMPLERS

Two different types of air particulate samplers are employed :

- single orifice impactors giving particle size information,
- "streakers", time sequence total filter samplers giving a continuous time record (fig. 1 et 2).

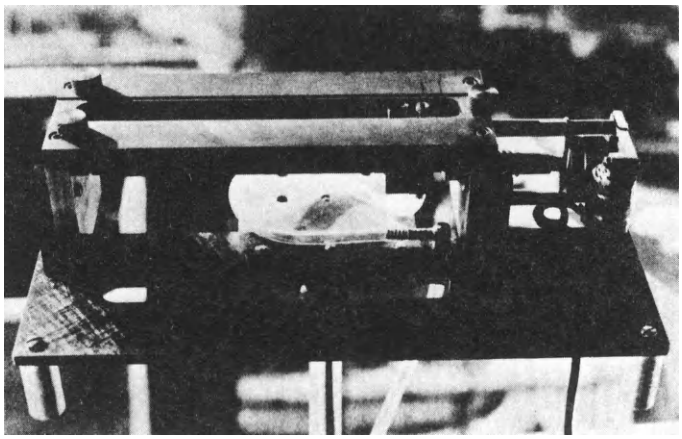

Fig. 1. View of the continuous filter sampler

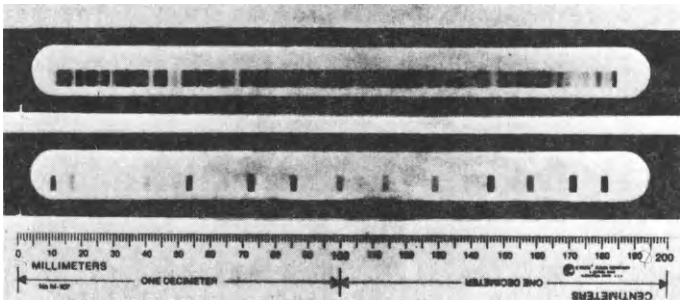

Fig. 2. Air particulate samples

We illustrate here our purpose in the case of streakers :

The aerosol sampling device collects particulate matter on a 0,4 μm pore diameter Nucleopore filter strip firmly attached to a 205 mm aluminium frame.This filter strip has a > 80 % particle collection efficiency down to 0.01 μm and below. A smooth, Teflon coated, 2 mm x 5 mm rectangular shaped sucking orifice is drawn by a clock motor at the rate of 1 mm/hr along the length of the filter strip. Ambient air is drawn through a 0,1 cm^2 area of the filter strip at the rate of 0,60 l/min. by a constant-volume pump. A single sampling frame can collect particulate matter in 2-hour increments for a total of one week unattended. Sample changing takes a few minutes. Because of the nature of the sliding orifice this aerosol sampling device has come to be called the "streaker". The device is positioned in the field so that the air is drawn upward from the underside of the filter, and large particles of about 30 μm and larger are thereby excluded.

ANALYSIS METHOD

The particulate matter on the filter strip is analysed for its elemental composition by proton induced X-ray emission (PIXE). This technique is a non-destructive, multi-element procedure in which protons excite the atoms of a sample, and the characteristic emitted X-rays are used to identify and to quantify the amount of each elements in the sample.

Excitation system

The FSU tandem Van de Graaf accelerator used for the PIXE analysis gives low energy (4 to 5 MeV) protons beams of 10 to 50 nano amperes. During analysis the sample frame is placed in an irradiation chamber at the end of the accelerator beam line as shown in figure 3.

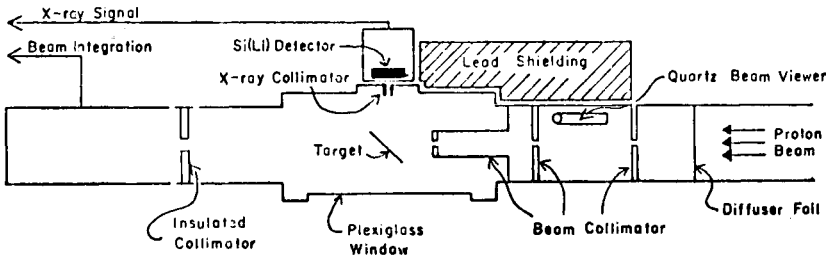

Fig. 3. Irradiation chamber

Samples can be positioned and exchanged without breaking the accelerator vacuum. The 0,10 cm^2 rectangular area beam transverses the thin sample and is absorbed in a Faraday cup connected to a beam integrator. Relative to the beam direction, the sample is generally oriented at 45° and the X-ray detector at 90°. This is achieved by investing a thin aluminium diffuser foil that speads out the beam.
Carbon collimators are then used to define the beam geometry. During analysis the streaker frame is moved automatically in discrete steps through the collimated proton beam.

Detector

A 80 mm^2 Si(Li) detector at a distance of 60 mm from the sample is used for characteristic X-ray detection.
The detector has a sensitive depth of 5mm and a resolution of 180 eV at 6 KeV.
A spectrum containing ten to fifteen elements ranging from silicon to lead may be accumulated in a multi-channel analyzer.

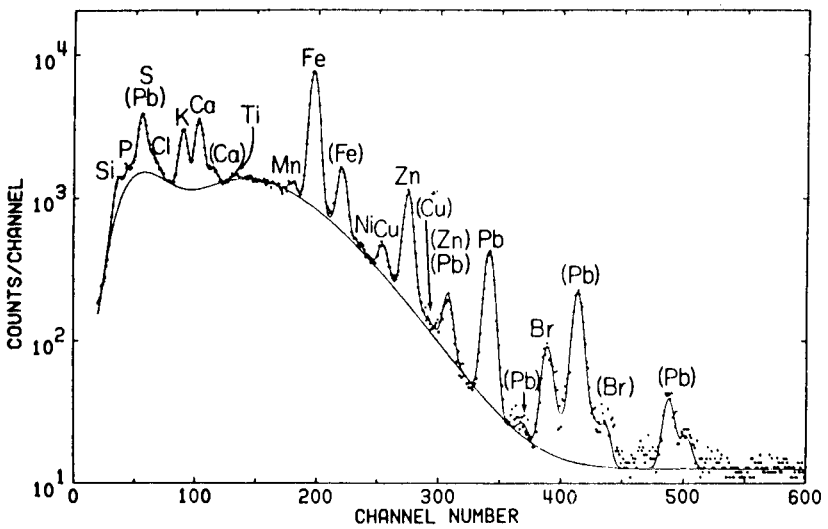

Fig. 4. PIXE spectrum of a streaker sample (10 mm^2 area)

Analysis program

The spectrum is stored on magnetic tape and later computer resolved using a non-linear least squares analysis. The analysis program (Kaufmann, Akselsson and Courtney, 1976) approximates the peak shape with an analytical function that consists of a Gaussian plus other components that effectively model the physics involved (i.e, Bremstrahlung from secondary electrons, Compton scattering within the detector, absorption within and outside of the target). Pulse height spectra are thus resolved yielding the individual elements by amount found in the sample

RESULTS

A total of 84 to 87 successive bombardments (depending on the sample) corresponding to a week of sampling time, usually takes less than 3 hours of beam time.

Minimum detection limits

Table 1 gives an axample of practical detection limits for sampling in a non-urban location. Naturally those values depend on the total composition of the samples.

Table 1 : Argonne III.(6-13 June 1976)

Maximum + and minimum - of analysed element concentrations. Total error and detection limits.

Elements	Extreme detected values. (ng/m^3)	Total error $\pm$ ng/m^3	Detection limits
Si	+ 3 740 - 324	1 156 163	203 199
S	+ 6 597 - 424	1 635 105	100 57
Cl	+ 157 - 58	37 22	57 56
K	+ 509 - 57	97 22	41 56
Ca	+ 3 476 - 338	36 68	653 50
Ti	+ 100 - 36	25 09	49 25
Mn	+ 225 - 41	37 16	33 37
Fe	+ 1 615 - 126	224 25	22 35
Zn	+ 1 161 - 28	168 13	21 25
Br	+ 396 - 55	69 13	74 25
Pb	+ 1 223 - 179	207 37	181 86

Results

Fig 5 shows the variation of K with time on one week period at Argonne (Illinois).

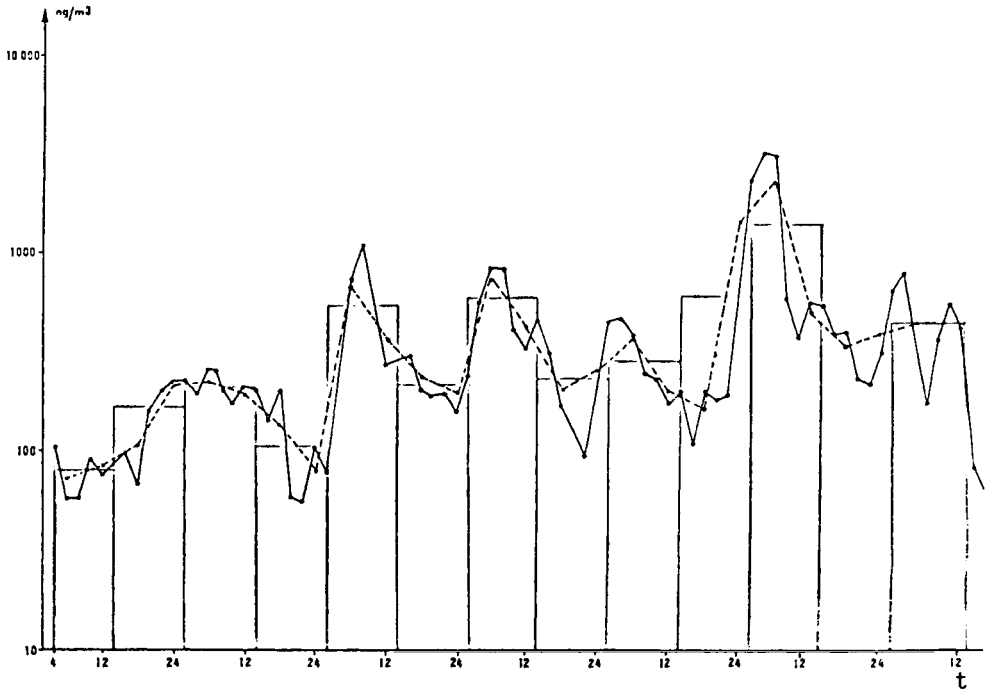

Fig. 5.Elemental concentration of K Vs time for the Argonne tower sampling site.

—— 2 hours time step
--- 6 hours time step
⌐¬ 12 hours time step

As shown, the tracking and monitoring of movements in terms of atmospheric particulate elements is critically dependent on the sampling device and on the associated analytical technique. A time dependent particulate sampler and a high resolution analytical procedure are essential with resolution down to at least from 2 hours to 4 hours in order to have desirable results. For the time period considered here, a 24 hours average particulate sample would have effectively obscured any gradients.

CONCLUSION

PIXE Analysis is becoming the most suitable method for routine analysis of atmospheric aerosols.

With the help of the Oceanography department of the Florida State University and with the collaboration of the "Laboratoire d'Analyse par Réactions Nucléaires C.E.N. France" we are working to set up a similar installation in France.

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Recent Advances in Surface Analysis of Environmental Particles

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ABSTRACT

The use of electron microprobe X-ray spectrometry, electron spectroscopy for chemical analysis, Auger electron spectrometry, and secondary ion mass spectrometry for performing surface analyses is described. Several examples of applications of these techniques to surface studies of environmental and source particulates are presented. It is apparent that many potentially undesirable species are enriched at the surfaces of anthropogenic particles and that most of these species are readily soluble in aqueous solution even though the bulk particulate material is essentially insoluble. It is concluded that surface analysis can provide valuable insights into the processes of formation and transformation of environmental particles and into their potential environmental impact.

Key words: Depth Profile, Flyash, Leaching, Particle, Surface Analysis

INTRODUCTION

Many of the processes which determine the chemical transformations of pollutant species, their adverse effects, and their transfer between environmental compartments take place at an interface or surface. For example, the so-called surface microlayer present on ocean and fresh waters is known to accumulate both organic and inorganic pollutants and to control their aquatic-atmospheric exchange (Suffet, 1977). Likewise, adsorption of pollutants onto airborne transport, deposition, reactivity, and potential toxicity (Linton and others, 1976; Novakov and others, 1974; Suffet, 1977). Interfacial phenomena are just as important in the biosphere where membrane transport characteristics can play a controlling role in determining pollutant toxicity (Luckey and others, 1975).

Conventional studies of environmental systems involve collection of bulk samples of air, water, soil, or of atmospheric and aquatic particles and determination of the average concentrations of pollutant species therein. While the utility of such measurements is beyond dispute, it must be stressed that they do not provide information about the identities and amounts of pollutant species which are present in the microscopically small regions which constitute environmental interfaces. It is the purpose of this article, therefore, to consider some of the methods which can be employed to obtain

information and provide an understanding of the behavior and effects of certain environmental pollutant species.

Specifically, attention is focussed on particulate materials whose surface chemistry is important for the following reasons:
1. A number of potentially toxic trace metal and organic species are highly enriched at the surfaces of many types of environmental particles (Davison and others, 1974; Linton, 1977; Linton and others, 1976, 1977; Luckey and others, 1975; Natusch and others, 1977; Novakov and others, 1974).
2. It is the surface of a particle which is directly accessible to extraction by aqueous leaching in the natural environment and by body fluids following inhalation or ingestion (Natusch and others, 1977).
3. It is the surface chemistry of a particle which governs its heterogeneous reactivity with gaseous or solution species (Linton and others, 1977; Natusch and others, 1977; Novakov and others, 1974).

Such considerations clearly point to the need for obtaining surface compositional information if the behavioral characteristics of particulate material are to be fully understood.

METHODOLOGY AND INSTRUMENTATION

Two types of analytical measurement are normally performed in surface studies of particulate material. These are:
1. Surface analysis to determine the elemental, and in some cases the molecular, composition of the outermost atomic layers.
2. Depth profile analysis to determine the variation of chemical composition with depth below the original surface.

Both types of measurement may be performed either on a single particle or on a field of particles although individual particle analysis requires microprobe capabilities. Such capabilities further enable generation of compositional maps which establish the lateral distribution of an element (Czanderna, 1975; Linton and others, 1978; McCrone and Delly, 1973).

From an analytical standpoint, there are four problems, not normally encountered in conventional analyses of bulk material, which are associated with surface measurements. The first is that quantitative analysis is extremely difficult due to the uncertainty in defining a precise analytical volume. The second involves the difficulty of obtaining a truly representative measurement since one commonly encounters considerable variations in sample composition even within a set of apparently similar particles. Thus, in achieving the specificity associated with characterization of a single particle one sacrifices statistical information unless a large number of particles are investigated serially or a field of particles is studied in a single measurement.

The third problem area is associated with sensitivity. Thus, surface analytical techniques must be extremely sensitive due to the very small analytical volumes sampled. Indeed, unless a given species is highly surface enriched, it is frequently undetectable unless the bulk concentration is greater than 0.1 to 1.0 percent. Finally, since surface analytical techniques involve bombardment with charged particles, there is a danger of changing the sample due to chemical reaction, selective volatilization of elements and migration in the solid. These last two artifacts are illustrated in Fig. 1 which shows the variations of Cl, Fe, K and S concentrations with time during bombardment with an electron beam. In general, it is difficult to obtain authentic depth

profiles of volatile species such as chlorine and mobile species such as the alkali metals.

The ESCA technique irradiates the sample with X-rays and records the energies of the emitted electrons. These energies provide information about valence states of major and minor elements (>1 percent atomic) present within ∿20 Å of the surface (Novakov and others, 1972). Depth profiling is achieved by etching the surface with an ion beam between analyses. The utility of ESCA for individual particle analysis is limited due to the difficulty of focussing X-rays to a beam diameter smaller than 1 mm, although recent advances (Hovland, 1977) indicate that lateral resolutions of ∿10 μm are feasible. Normally, the sensitivity of ESCA is insufficient to enable observation of trace constituents unless considerable surface enrichment is encountered (Evans, 1975).

In AES the emission of Auger electrons is stimulated by bombarding the sample with a beam of electrons. The energy of the secondary Auger electrons is characteristic of the emitting element and is determined using a cylindrical mirror analyzer (Fig. 2). Spectra are recorded in the first derivative mode to discriminate against a background of inelastically scattered electrons. Elemental detection limits lie in the range 0.1 to 1.0 percent (atomic) within the analytical volume (depth ∿20 Å) and decrease with increasing atomic number as competitive X-ray emission becomes more efficient. Depth profiling is achieved by etching the sample surface with an ion bean (normally Ar^+) as in ESCA. Most AES spectrometers possess microprobe capabilities with incident beam diameters of 1-5 μm.

In SIMS the sample is bombarded with a stream of ions (most commonly negative oxygen ions) and surface material is physically removed. About 1 to 10 percent of the sputtered material is in the form of secondary ions which are mass analyzed by a conventional mass spectrometer (Fig. 3). The ion microprobe (Bakale and others, 1975) represents a special configuration of SIMS in which the primary ion beam can be focused to a diameter of about 3 to 5 μm. Both individual particle analysis and elemental mapping capabilities are thus available and depth profiling constitutes an integral part of the process of secondary ion generation. A major advantage of SIMS is its extremely high sensitivity with elemental detection limits ranging from 10^{-2} to 10^{-6} percent atomic depending upon the element and the primary ion employed. Typically, it is possible to observe as little as 1 μg/g in the analytical volume (Evans, 1975) thereby enabling studies of species present at trace levels.

SIMS is, however, subject to several types of interferences and artifacts. In particular, spectral interferences from molecular and multiply charged ions make the high resolving power of a double focussing mass spectrometer desirable (Linton and others, 1977; Natusch and others, 1977). Also, volatilization losses and migration of sample ions under the influence of the primary ion beam can give rise to spurious depth profiles as illustrated for an electron beam in Fig. 1. Such effects are often difficult to identify in SIMS since removal of surface material is an integral part of the detection process.

The technique of Time Resolved Solvent Leaching (TRSL) does not require expensive instrumentation and is, at least potentially, available to most moderately well equipped laboratories. For the TRSL approach a solvent is passed continuously through a very shallow bed consisting of the particulate sample. Aliquots of the emerging leachate are then analyzed for both cationic and anionic species. (In our case, cationic analyses are performed by atomic

adsorption spectrometry, plasma emission spectrometry, or differential pulse anodic stripping voltammetry. Anionic analyses are performed by ion chromatography.)

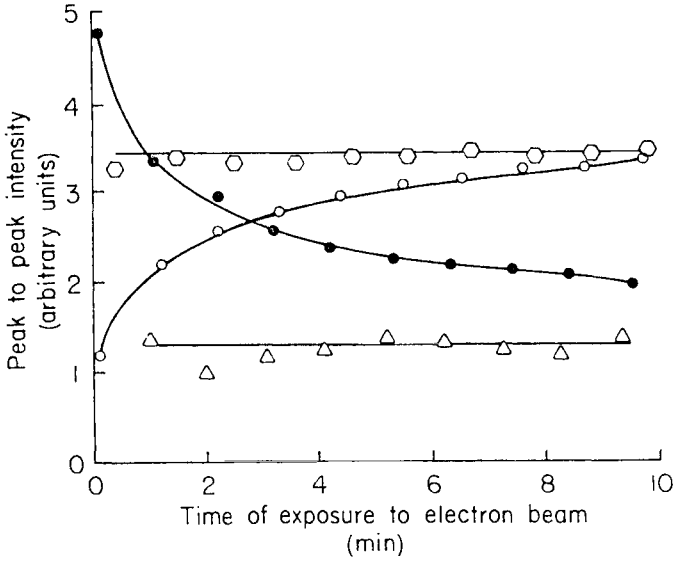

Fig. 1 Dependence of signal intensity on time during electron bombardment in Auger Electron Spectroscopy (Cl ○; Fe △; K ⬡; S ○).

At the present time there exist a large number of analytical techniques which can be employed for surface analyses (Czanderna, 1975). However, those which are most used, and which are discussed herein, are electron microprobe X-ray emission spectrometry (EMP), electron spectroscopy for chemical analysis (ESCA), Auger electron spectrometry (AES), and secondary ion mass spectrometry (SIMS). In addition, a new approach to surface analysis called Time Resolved Solvent Leaching (TRSL) is introduced as a potentially universal approach to surface analysis.

The electron microscope and microprobe bombard the sample with a focussed beam of electrons which stimulate emission of X-rays characteristic of the elements present. The technique is useful for analyses of individual micrometer-size particles and has a lateral and depth resolution of about one micrometer (μm) determined by the X-ray emission volume (Armstrong and Buseck, 1975; McCrone and Delly, 1973). Surface analysis capabilities are thus poor since the depth resolution is very much greater than the thickness of the surface predominance can be obtained only by varying the energy (depth penetration) of the electron beam, by analyzing before and after ion etching, or by comparing elemental ratios obtained by bulk and EMP analyses (Czanderna, 1975; Linton and others, 1976, 1978).

The leaching process is described by the equation

$$P(M_i)_{m_{ij}}\ (A_j)_{a_{ij}} \underset{(k_{-1})_{ij}}{\overset{(k_1)_{ij}}{\rightleftharpoons}} P + M_{ij}\ M_i^{a^+_{ij}} + a_{ij}\ A_j^{m^-_{ij}} \qquad (1)$$

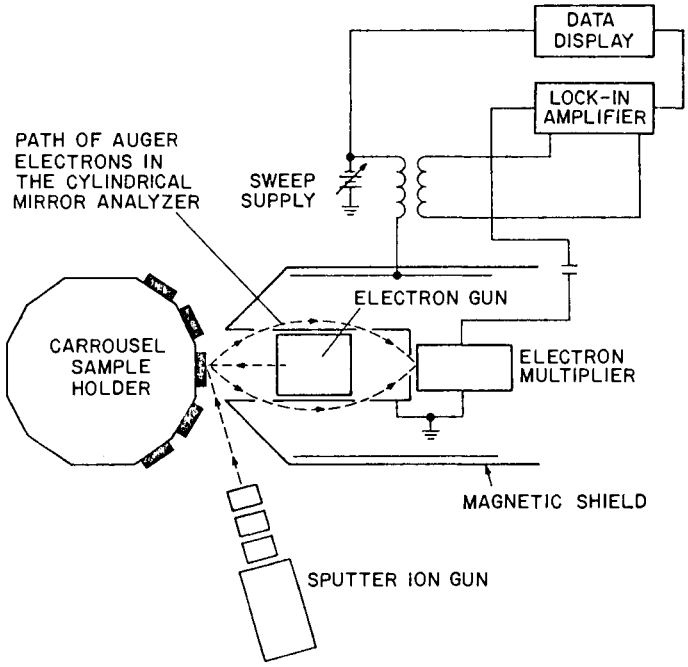

Fig. 2. Schematic diagram of a conventional Auger Electron Spectrometer.

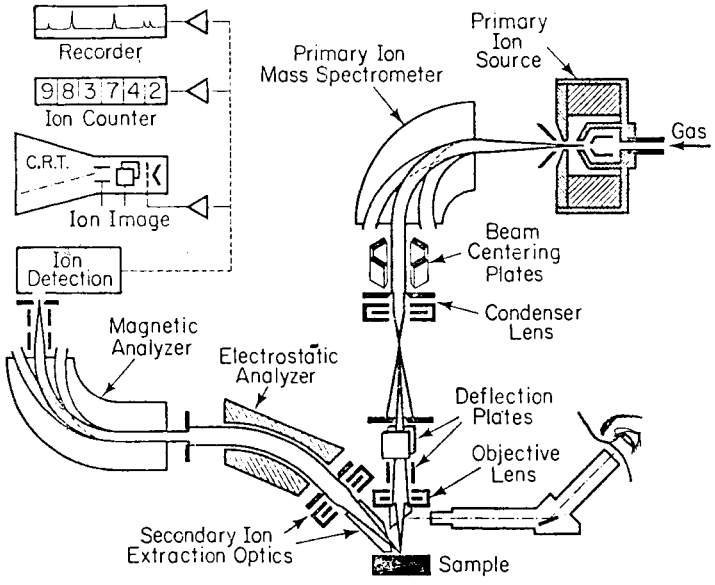

Fig. 3. Schematic diagram of an Ion Microprobe Mass Spectrometer.

where the cationic species M_i and the anionic species A_j have a valence of m_{ij} and a_{ij}, respectively. The dependence of ionic (in this case, anionic) concentration in the leachate on time is given by

$$\frac{d[A_j]}{dt} = [P] \sum_i (k_1)_{ij}\, a_{ij}\, (\theta_{ij}(r) - \left(\frac{[M_i]^{m_{ij}}[A_j]^{a_{ij}}}{(K_{sp})_{ij}} \right) \qquad (2)$$

where P = the particle surface area
M_i, A_j = cations and anions respectively
m_{ij}, a_{ij} = stoichiometric coefficients
$(k_1)_{ij}$ = forward dissolution constant
$\theta_{ij}(r)$ = fractional surface coverage of ij^{th} component at radius r
$(K_{sp})_{ij} = (k_1)_{ij}/(k_{-1})_{ij}$

It can be shown that, when the reverse process in equation (1) is insignificant, as is the case for very high solvent flow rates or when the solvent approximates an infinite ionic sink (e.g. for an acidic or chelating solvent), then the term

$$\left(\frac{[M_i]^{m_{ij}}[A_j]^{a_{ij}}}{(K_{sp})_{ij}} \right)$$

in equation (2) becomes insignificant so that the time profile dA/dt is directly proportional to the depth profile of the species A_j (of M_i). Thus, meaningful depth profiles can be established such as are illustrated in Fig. 4. Quantitative estimations of surface concentrations rely, however, on evaluation of the dissolution rate constants, k_{ij}, and the solubility products, K_{ij}^{sp}, in equation (2). The advantages of this approach are:

1. It enables utilization of the most sensitive analytical methods for determining all species entering solution.
2. It provides information both about the surface distribution of a species and about its identity.
3. It establishes which chemical species can be mobilized in solution and can thus have an environmental or toxicological impact.
4. It is applicable to organic species which are normally removed from surfaces under the high vacuum conditions associated with instrumental techniques.
5. It is inexpensive, thereby making the field of surface analysis available to most scientists.

Of the above techniques, AES and SIMS are currently most useful for surface analysis and depth profiling studies due to their sensitivity and good lateral and depth resolution. ESCA, however, has the important advantage of providing information about the identity of molecular species present. In all cases difficulties are encountered in establishing even semiquantitative depth scales. This is normally approached by calibrating the rate of removal of surface material against that obtained for a standard having a surface layer of known thickness. The main problem, however, lies in matching the matrix composition of the standard to that of the material being studies. In the

case of environmental particles matrix and surface compositions are not, in general well defined.

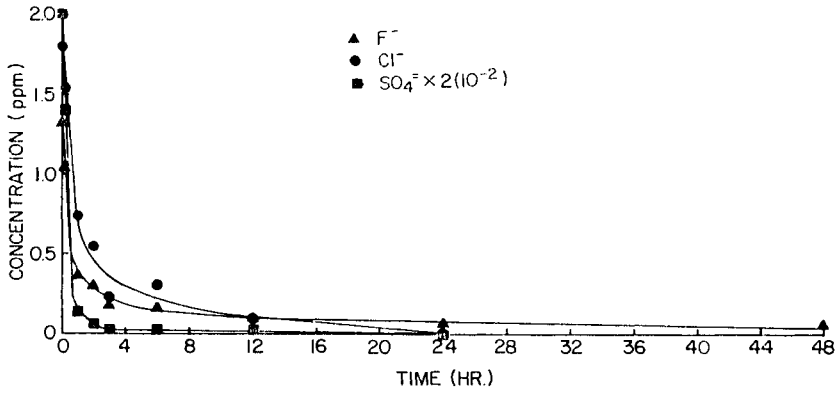

Fig. 4. Time Resolved Solvent Leaching depth profiles of chloride, fluoride and sulfate anions associated with coal fly ash.

STUDIES OF ENVIRONMENTAL PARTICLES

Most anthropogenic particulate matter which enters the environment contains higher specific concentrations (μg/g) of several potentially hazaradous trace elements than are normally found in natural crustal dusts, soils, and sediments (Butcher and Charlson, 1972; Natusch and others, 1978). In many cases these elements, which include As, Cd, Co, Cr, Mn, Ni, Pb, S, Sb, Se, Tl, V, and Zn, are preferentially associated with particle surfaces as a result of condensation from the vapor phase or adsorption from solution (Davison and others, 1974; Linton and others, 1977; Natusch and others, 1978). Such surface association is of special environmental significance since it leads to preferential enrichment of small particles which are least effectively collected by pollution control devices, which have long atmospheric and aquatic residence times, and which can deposit in the pulmonary region of the human respiratory tract when inhaled (Cunningham and others, 1974; Davison and others, 1974; Kaakinen and others, 1975; Lee and others, 1975; Lee and von Lemden, 1973; Natusch and others, 1974).

Several of the surface analytical techniques described earlier have been used to study airborne particles and particles derived from specific sources. The results of such studies are presented here to illustrate the types of information which can be obtained.

Airborne Particles

The most definitive surface studies of composite airborne particulate matter have employed ESCA and have emphasized the capability of this technique to identify the chemical compounds present. Sensitivity limitations do not enable detection of trace species; however, it has been established that the elements C, N, Pb and S are surface predominant (Greiger, 1976). Most aerosol sulfur is in the form of sulfate and most nitrogen is present as the ammonium ion. Quantitative measurements of these species indicate their

association as ammonium bisulfate and sulfate. In addition, however, Novakov and others have identified substantial amounts of amine nitrogen (Chang and Novakov, 1973; Novakov and others, 1972, 1974).

ESCA has proved especially useful for following the surface chemistry of sulfur and nitrogen compounds with changes in meteorological conditions (Greiger, 1976). Thus, Novakov and others (1974) and Chang and Novakov (1973) have demonstrated the catalytic role of atmospheric carbon (soot) particles in heterogeneous reactions involving NH_3, NO, and SO_2.

Coal Fly Ash

Fly ash derived from combustion of coal in a conventional power plant is composed primarily of an impure aluminosilicate glass together with small amounts of several crystalline minerals (Linton and others, 1976, 1977; Natusch and others, 1977). Only about 1 to 3 percent of the bulk material is soluble in water (Nautsch and others, 1977). Depth profiling studies using EMP, AES, and SIMS have established that a number of elements including C, Cr, K, Mn, Na, Pb, S, Tl, V, and Zn are substantially surface enriched whereas Al, Ca, Fe, Mg, Si, and Ti are not (Linton, 1977; Linton and others, 1976, 1977). This observation supports the hypothesis (Davison and others, 1974) that the more volatile elements, or their compounds, are vaporized during combustion and then condense on the surfaces of co-entrained fly ash particles at lower temperatures.

Depth profiling studies of fly ash have also demonstrated the utility of employing instrumental techniques in conjunction with solvent leaching to remove soluble surface material. An example of this approach is presented in Fig. 5 for the elements Pb and Tl. The results show that extraction of fly ash with water or dimethylsulfoxide removes the surface layer of both elements. Determination of the amounts of Pb and Tl in solution then enables estimation of the amounts present in the surface layer. If one assumes this layer to be 300 Å thick then one obtains average concentrations of 2700 μg/g for Pb and 920 μg/g for Tl in the surface layer as compared to bulk particulate concentrations of 620 μg/g and 30 μg/g for these elements, respectively.

Solvent leaching can also provide some insight into the chemical forms of elements present. For example, although AES and SIMS indicate little surface enrichment of iron, aqueous leaching rapidly removes this element from the surface region thereby indicating its presence there in a readily soluble form. Furthermore, comparison of the leaching and depth profiles of K, Fe, Na, and S suggest that these elements may be associated in the surface layer--possibly as alkali-iron sulfates (Reid, 1971). Further support for the existence of simple and/or complex sulfates if provided by ESCA studies which show that the oxidation states of Fe and S in the surface region are +3 and +6 respectively (Small, 1975).

From the standpoint of environmental pollution these results have a number of ramifications:

1. Since a number of toxic elements are highly enriched in soluble form on fly ash surfaces, their potential toxicological impact may be significantly greater than that predicted from bulk analysis. Such enrichment will be greatest in small, pulmonary depositing particles (Davison and others, 1974; Natusch and others, 1974) and will give rise to high localized trace metal concentrations in the micro-regions of the lung where the particles are deposited.

2. The surface enrichment of alkali metals, which determine the resistivity of fly ash particles (Bickelhaupt, 1974; Bickelhaupt, 1975), will enhance the ability of the particle surface to hold a charge and, in turn, improve the efficiency of electrostatic precipitation (Kanowski and others, 1977).
3. The presence of acid sulfates on fly ash particle surfaces may, of itself, enhance their toxicity (Finklea and others, 1977).
4. The existence of sulfate on fly ash surfaces indicates the ability of fly ash to function effectively as a catalyst for the heterogeneous oxidation of SO_2 (Judeikis, 1977). Fly ash may, therefore, be important in determining the extent of SO_2 to sulfate conversion in power plant plumes (Lusis and Wiebe, 1976).

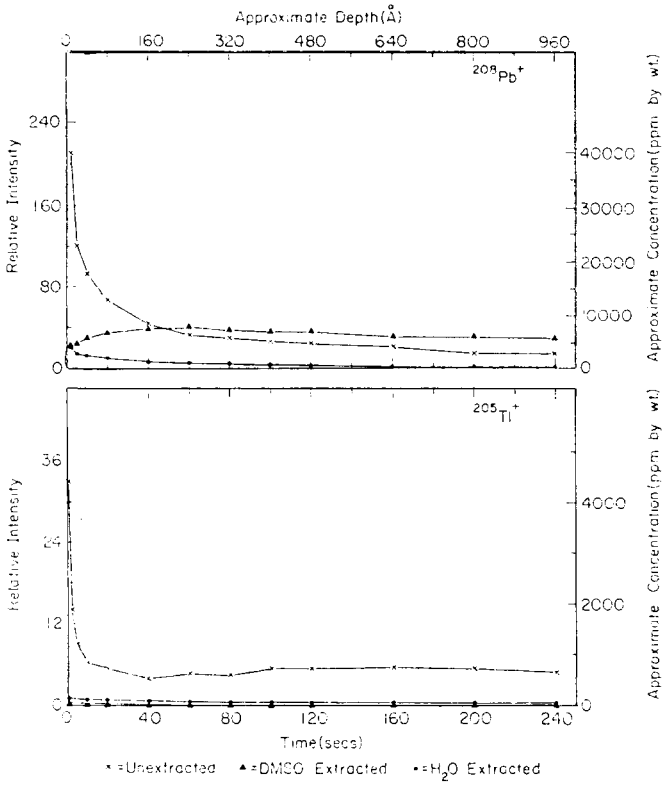

Fig. 5. Depth profiles of Pb and Tl in coal fly ash obtained by Secondary Ion Mass Spectrometry before and after extraction with water and with dimethylsulfoxide.

Automobile Exhaust Particles

Scanning electron microscopic investigations show that automobile exhaust particles occur in two distinct morphological forms (Boyer, 1973; Linton and others, 1978; Loh, 1975). The first involves quite large particles (>10 μm cross-section) of irregular size (Olson and Skogerboe, 1975). Individual (EMP) particle analyses indicate that the predominant elements are Fe, Pb, Br, Cl, and S together with highly variable amounts of Ca and Si. Elemental depth

profiles obtained using AES and SIMS indicate that Pb, Br, Cl and S are enriched at the particle surfaces but that Fe is not (Fig. 6 and Fig. 7). The region of surface predominance is estimated to extend less than 1000 Å into the particle interior. It is also interesting to note that SIMS shows the trace elements Cr, Ni, and Tl to be highly surface enriched (Linton and others, 1977).

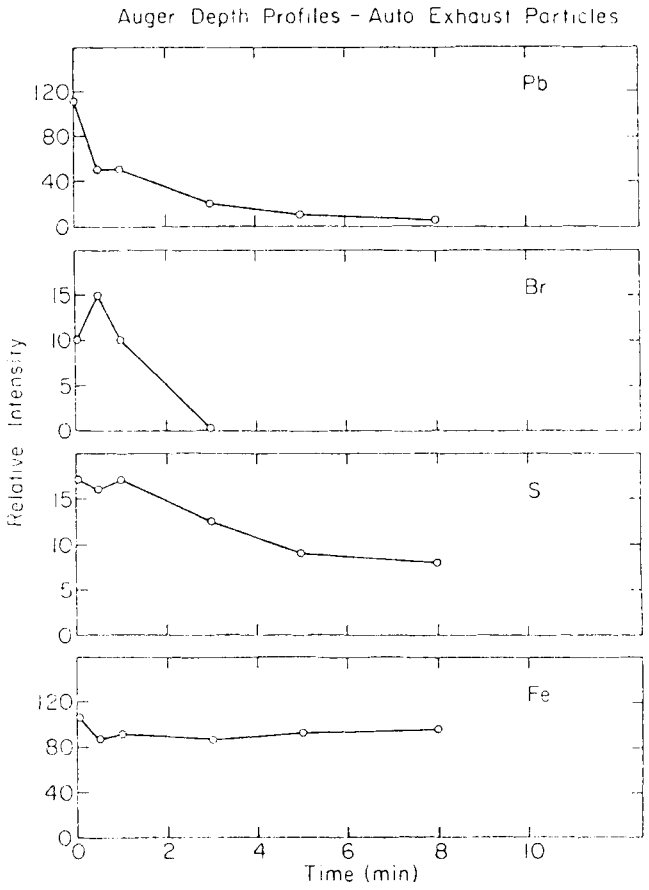

Fig. 6. Depth profiles of Pb, Br, S, and Fe in automobile exhaust particles obtained by Secondary Ion Mass Spectrometry.

The second particle type involves small (<1 μm diameter) spherical particles which are composed almost exclusively of Pb, Br, and Cl but which contain no Fe. Surface analysis of such small particles is extremely difficult; however, preliminary indications are that none of the constituent elements are surface predominant.

These findings suggest that the two types of particles present in automobile exhaust emissions are formed by quite different processes. The surface predominance of Pb, Br, Cl and S on large particles is attributed to deposition

of volatile lead and sulfur species (probably PbBrCl and SO_2) onto the surfaces of refractory iron-containing particles as the temperature decreases in the automotive exhaust system (Bomback and others, 1975). The iron-rich host particles are probably derived from ablation of iron from the exhaust system. The small amounts of Cr, Ni, and Tl observed probably originate as impurities introduced with the lead gasoline additive or, in the case of Cr and Ni, are derived from engine wear. In either event, their surface predominance strongly indicates their vapor phase history.

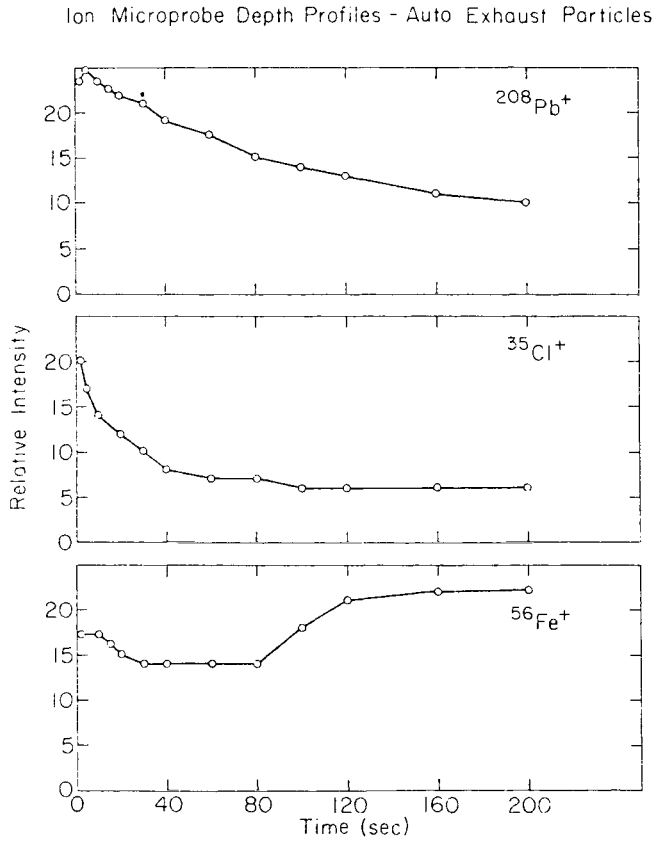

Fig. 7. Depth profiles of Pb, Cl and Fe in automobile exhaust particles obtained by Secondary Ion Mass Spectrometry.

The homogeneous nature of the small spherical exhaust particulates suggests that their formation occurs by a nucleation process in which PbBrCl condenses to form quite pure molten droplets when the temperature of the exhaust system falls below the saturation point. There is, however, little supporting evidence for this suggestion.

Open Hearth Furnace Dusts

Baghouse dusts derived from the open hearth smelting of iron provide an interesting example of the operation of several of the artifacts associated with instrumental surface analysis. These dusts consist of three morphological forms: large (>60 μm cross-section) irregular slag particles; large spheres (>90 μm diameter) of glassy appearance; and small (<1 μm cross-section) grains of reddish-brown Fe_2O_3 which adhere strongly to the large particle surfaces.

EMP analysis shows that the predominant elements in the slag are Al, Ca, K, Mg, and Si, whereas Ca, Cl, Fe, K, S, and Zn are predominant in the glassy spheres. Iron and oxygen, on the other hand, are apparently depleted near the surface.

It is apparent from visual observation of these dusts following EMP and AES analysis that considerable sample modification occurs as a result of both electron and ion bombardment. In particular, exposure to constant electron bombardment in AES results in changes in the intensity of the Cl and S signals as illustrated in Fig. 1. These changes are attributed to volatilization in the case of Cl and to migration in the case of S. Under high electron beam intensities Si is observed to decrease with time and K to increase indicating additional disturbance of the surface chemistry (Harris, 1968). These effects lead one to suspect the validity of depth profile analyses and it is necessary to perform several tests to distinguish real effects from artifacts. Such tests include variation of electron beam intensity, minimization of irradiation time, solvent leaching, and sequential heating of the sample in an inert atmosphere. It is interesting to note that, as in the case of coal fly ash, the surface associated elements (in this case Ca, Cl, K, P, Pb, S, and to some extent, Zn) are quite soluble in water even though the bulk matrix is essentially insoluble.

CONCLUSIONS

Consideration of the foregoing examples indicates two important points. First, a great deal of new information about the physical and chemical characteristics of particulate surfaces can be obtained using the techniques discussed. Secondly, most of the insights provided are highly speculative. It is suggested, therefore, that surface analytical results should be utilized primarily for formulating hypotheses about probable surface chemistry, mechanisms of particle and surface layer formation, and potential environmental effects. Such hypotheses are then best checked by additional independent experiments in which surface analysis may or may not be used for monitoring purposes.

Further development of environmental surface analysis seems likely to proceed via two distinct pathways. The first involves combined (ideally simultaneous) use of several techniques. For example, combined use of SIMS and AES or ESCA provides the capability of observing both material removed from a surface (SIMS) and that freshly exposed (AES or ESCA) as the analysis proceeds. Such an approach would help in identifying changes which occur as a result of charged particle bombardment. The second approach involves the use of time resolved solvent leaching (TRSL) which provides the advantages of high sensitivity, low cost of operation and applicability to organic materials.

Overall, therefore, it appears that surface analysis has provided, and will continue to provide, valuable insights into the characteristics, formation, reactivity, and eventual impact of environmental particles. Perhaps above

all it has indicated the very real significance of surfaces in determining a wide variety of environmentally important processes.

ACKNOWLEDGEMENTS

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Identification of Inclusions and Particles by Raman Microprobe

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ABSTRACT

A new microanalytical technique based upon Raman scattering is presented. This non destructive technique founded upon a molecular property brings us information about the polyatomic structures present in a microsample. The Raman microprobe MOLE perfected in our laboratory does not only give Raman spectra of microsamples (about one µm) but also the microscopic distribution of the heterogeneous sample components. Many applications illustrate inclusions and particles *in situ* analysis in geological, biological and synthetic materials.

KEYWORDS

Raman effect, Microanalysis,Raman Microprobe, Micro-chemistry, particulate matter.

INTRODUCTION

The purpose of this paper is to present the microanalytical Raman techniques perfected and developped in our laboratory and to demonstrate their interest by presenting some results we obtained when analyzing inclusions and particles inside geological, biological and synthetic materials.

For twenty years numerous analytical techniques (electronic microscope and microprobe, ionic microprobe...) have been created that permit the determination of elemental composition of samples in the micrometer size range. But these techniques based upon atomic properties only directly bring us information about the polyatomic structures present in the sample.Unfortunately during the same time, the techniques that provide data on molecular structure (X- ray diffraction, neutron diffusion, N.M.R., vibrational spectroscopy IR and Raman) have not been extended to the microanalysis. However with the laser advent which permits to concentrate the energy of light beam into a very small volume, Raman spectroscopy appears to be a choice method for the analysis of very small amount of matter.

Raman effect relates to the change of frequency observed when a monochromatic light beam (ν_0 frequency) is scattered by polyatomic molecules. The scattered light contains near the ν_0 frequency radiation (Rayleigh diffusion without change in frequency) much weaker radiations with $\nu_0 - \nu_i$ frequency (Stokes lines) and $\nu_0 + \nu_i$ frequency (Anti Stokes lines). All these lines form the Raman spectrum. The ν_i frequencies (expressed in wave number $\bar{\nu} = \nu/c$ cm^{-1}) characterize the scattering media and are independent of the incident radiation frequency. They correspond to the frequencies of the atoms oscillations in polyatomic structures. They are directly read on Raman spectrum recording which only consists in the Stokes part (the more intense) and whose origin is the ν_0 frequency . Raman spectrum can be used to characterize and identify the chemical species and to precise their structure.

Raman analysis is relatively rapid (spectrum recording takes several tenth of minutes), non destructive and may be conducted in air as well as under a controlled atmosphere without special preparation of the sample. These facts have lead some workers to consider the possibility of using Raman spectroscopy as a microanalytical tool.

In 1973,Hirschfeld (1973) was the first to speak about the possibility to obtain Raman spectra from micron particles. Then Rosasco and co- workers (1975) at the NBS (USA) and independently Delhaye and Dhamelincourt (1975) in Lille have studied microanalytical techniques founded upon Raman scattering. In Lille a Raman microprobe has been realized (Dhamelincourt, 1977a, 1977b) that does not only give Raman spectra of microsamples but also the microscopic distribution of the heterogeneous sample components. This instrument is now commercialized by Jobin- Yvon optical systems under the name of MOLE (Molecular Optic Laser Examiner).

RAMAN MICROPROBE MOLE

MOLE reunits in the same instrument : a conventional optical microscope (with bright and dark field illumination), an optical filter possessing a very low stray light level (with two concave holographic gratings) and a monochannel or multichannel detection device. This association permits different operating modes but two modes are particularly used :

Punctual Illumination with Monochannel or Multichannel Detection

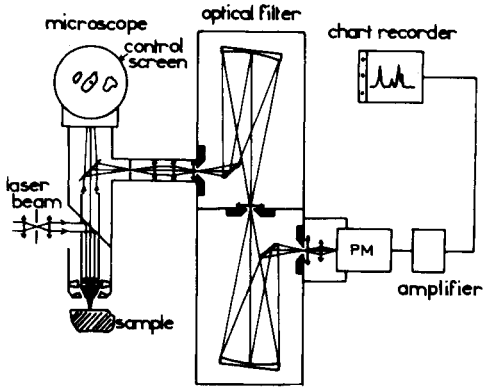

Fig.1. Punctual illumination mode

In this case (Fig. 1) when using bright field illumination system of the microscope, the same microscope objective (50x, N.A 0.85 ; 100x, N.A 0.90) is used to focus the laser beam (into a spot of about one micron) on the component to be identified and to collect the scattered light at the focus point. The scattered light is sent on the entrance slit of the optical filter which can work as a Raman microspectrometer when the detector is a photomultiplier followed by an amplifier and a chart recorder (monochannel detection) or a Raman microspectrograph when the detector is an intensifier phototube followed by a low level TV camera (S.I.T. or S.E.C.), in this case the spectrum is visualized on a TV monitor or a C.R.T. screen.

The depth of field i.e. the thickness of the region which is analyzed depends on the resolution used for recording the spectrum. By adjusting the entrance slit width close to the image diameter of the focus point on the slit, the depth of field can be reduced to a few microns. Thus only some μm^3 of matter are analyzed.

Global Illumination and Imaging System

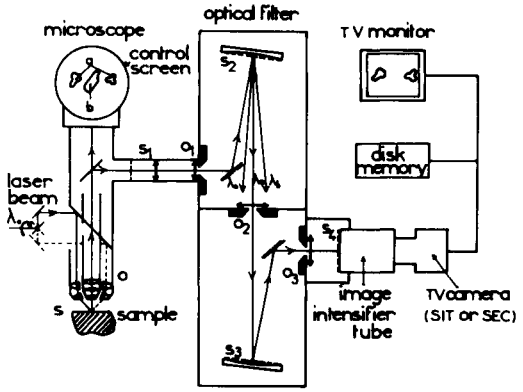

Fig.2. Global illumination mode

The sample is globally illuminated with a rotating laser beam feeding the objective annular illuminator (dark field illumination device). In this case (Fig. 2) the aperture of the microscope objective (O) is optically conjugated with the three slits (O_1, O_2, O_3) of the optical filter and the image (S_1) of the sample (S) given by the objective is formed on the gratings (S_2, S_3) then through the exit slit (O_3) on the photocathode of the intensifier tube (S_4) . By selecting in the Raman spectrum a radiation characterizing one peculiar component of the sample ("a" for instance) and by tuning the optical filter to this frequency a micrographic image giving the distribution of this component is formed on the photocathode of the intensifier tube and appears on a TV monitor. The spatial resolution of the image is about 1 µm . According to the nature of the sample and the problem to solve it is possible to mix the different systems of sample illumination and detection. For instance global illumination can be used with the monochannel detector or the punctual illumination with the imaging system.

ANALYTICAL APPLICATIONS OF RAMAN MICROPROBE TECHNIQUES

Many problems were submitted to us. In order to illustrate the potentiality of the method to adapt at various problems we have choosen several examples intotally different domains

Geological Samples : Analysis of Fluid Inclusions in Minerals

In most cases mineralogic reactions in the earth'crust occur in the presence of a fluid phase which may be trapped in the crystals defects. These fluid inclusions are real witness of the genesis of minerals and their composition are of great interest for the geochimist.

Microthermometry, chemical analysis of dissolved ions in the aqueous phase, gas analysis (by gas chromatography and mass spectrometry) are the mains techniques for studying these inclusions. However these techniques (excepted microthermometry) which require the sample grinding are destructive so that the chemical composition of the inclusion (particularly the phase gas) may be altered with regard to the actual composition before extraction.

A great improvment in the chemistry studying of fluid inclusions is provided using Raman microprobe that allows to analyse ponctually, *in situ*, without destruction, gas, liquid or solid in an individual inclusion within transparent media

Analysis of gas inclusions in the N_2 - CO_2 system. These inclusions (Fig. 3)

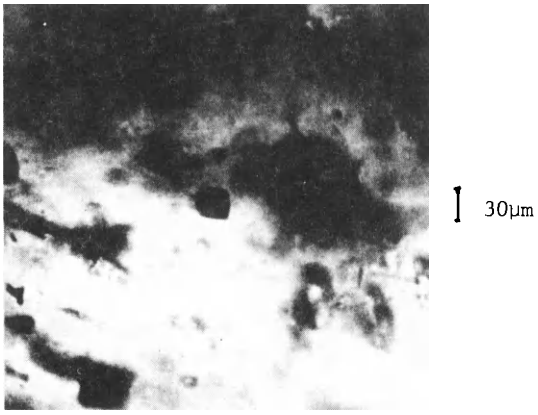

Fig.3. N_2- CO_2 inclusion

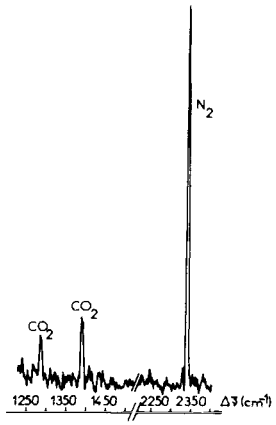

Fig.4. Raman spectrum of N_2- CO_2 inclusion

are found inside host quartz and dolomite from Central Tunisian diapirs. Raman microprobe investigation shows fossil fluids of variable ratio in the N_2- CO_2 system (Fig. 4). This result agrees with microcryoscopic data (Guilhaumou and others, 1978) but Raman analysis supplies more information since the ratio N_2/CO_2 can be reached directly from vibrational spectrum and cross- sections of these gas. This ratio lies between 0.5 and 9 according to the inclusion.

Hydrocarbon inclusions. In the same samples (quartz and dolomite) some monophase liquid inclusions containing aliphatic or aromatic hydrocarbon have been disclosed using Raman microprobe (Fig. 5 and Fig. 6)

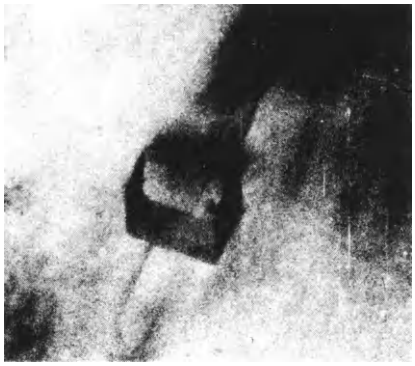

Fig.5. HYdrocarbon inclusion

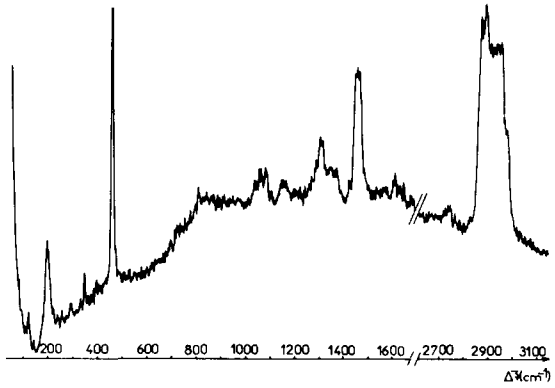

Fig.6.Raman spectrum of hydrocarbon inclusion

The both presence, inside the same crystal of these different inclusions(hydrocarbon and nitrogen) associated to the detection of oil field superficial signs in the same territory of Central Tunisian let suppose that this nitrogen has an organic origin.

Another interesting application is the measurement of $^{13}C/^{12}C$ ratios in liquid gas CO_2

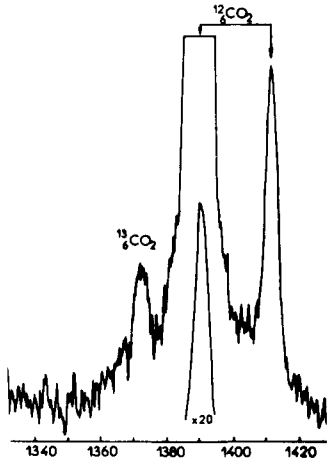

Fig.7. Raman spectrum of gas phase in triphase inclusion ($CO_2\ell$,CO_2g,$H_2O\ell$)

The spectrum reported on the Fig.7 corresponds to CO_2 gas phase within triphase ($CO_2\ell$, CO_2 g, H_2O ℓ) in a quartz from Camperio (Switzerland)[1]. The characteristic lines of $^{13}CO_2$ and ^{12} CO_2 appear respectively at about 1370 and 1390 cm^{-1}. The ratio of their intensity provides an evaluation of the $^{13}C/^{12}C$ ratio and by calibration with a CO_2 standard the ^{13}C value can be estimated with $\pm$ 15%

[1]Geological samples are from FOTY B.,C.R.P.G. Nancy (France).

relative incertainty.

MOLE is also used with success for studying precious stones, different gems and their inclusions. Their idendification permits to authenticate them and go back to their geographical origin (Dhamelincourt, Schubnel, 1977; Dele, Dhamelincourt, Schubnel, 1978).

Biological Samples

As recent works (Ballan- Dufrançais , Truchet, 1977) showed it Raman microprobe analysis of cells and tissues concretions (mineralized or not) is possible. Thus guanine has been detected inside fish and spider tegument. Likewise xanthine, uric acid, sodium and potassium urates have been identified *in situ* in concretions of the snail digestive gland and inside spherocrystals from utricules and fat body of the Blatella Germanica (cockroach) (Fig.8, Fig. 9). Before the advent of Raman microprobe techniques such studies require greater quantities of entire organs

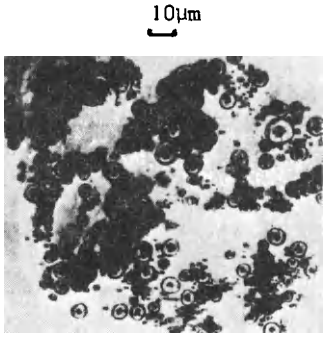

Fig.8. Spherocrystals in fat body.

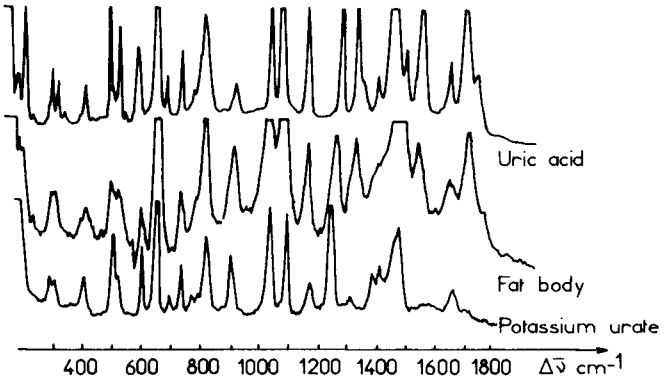

Fig.9. Raman spectra of spherocrystals

The following examples illustrate other applications upon biological samples.

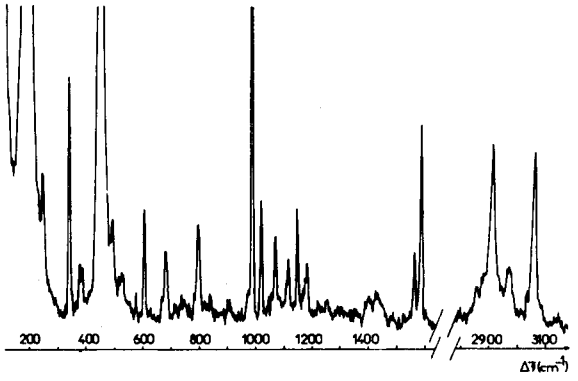

Fig.10. Raman spectrum of an aromatic compound

As reported spectrum (Fig.10) shows it ,other organic substances can be identified inside histologic sections of alive tissues without fluorescence disturbance. This spectrum relates to an aromatic compound.

Foreign body in fish liver[2]. We have detected carbon particles in a fish liver (Fig. 12). The reported spectra (Fig. 11) correspond to different graphite species more or less cristallized and we established the more crystallization state increases the more the two characteristic lines are resolved. Therefore we concluded that the analyzed particle is not crystallized according to its spectrum.

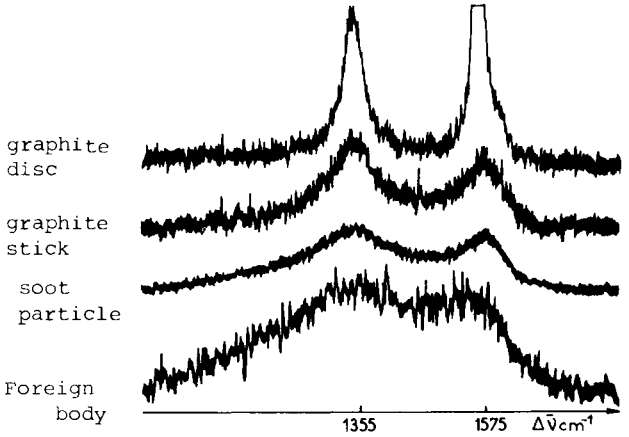

Fig. 11. Raman spectra of different graphite species

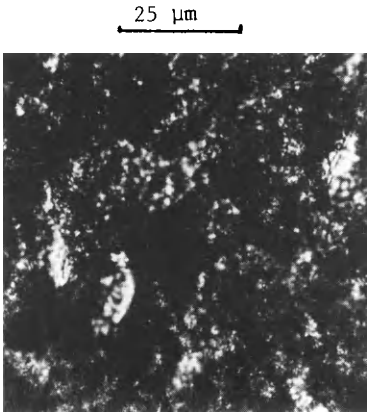

Fig. 12. Foreign body in fish liver

Investigation of Defects in Synthetic Materials.

Formation of defects is a problem currently encountered in the commercial production of materials . Characterization of bubble- like and haze- like defects inside synthetic fibers or films which are at the origin of their breaking have been successfully solved by Raman microprobe technique. Generally these defects were found to be local differences of crystallinity or local concentration of copolymers in terpolymers. Beside these defects many inclusions were also observed and analyzed in synthetic materials. They are simply atmospheric dust particles (α SiO_2, $CaCO_3$...) embedded in the polymer during the manufacturing process.

Defects identification inside PET used in different industrial domains. These defects appear as about a few tenth microns inclusions and may induce various disturbances according to the use. The defects spectrum recorded with punctual illumination mode characterizes the inclusion. Such a spectrum is presented on Fig.13.

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[2] This work has been realized in collaboration with Pr. Vandorpe and M.C. Dhamelincourt.

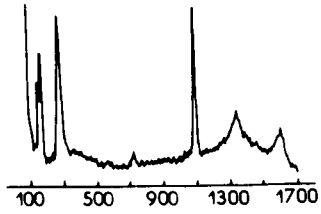

Fig.13. Raman spectrum of defect in PET

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Contributions to the Development of Natural Urban Particulate Matter Standard Materials

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ABSTRACT

Sampling, recovery, chemical analysis and characterization of fine ambient urban particulate matter which can be found abundantly deposited in filter systems of large scale air conditioning installations will be reported in this paper.

Using these non costly sources, sufficient amounts of fine dust material have been collected at several locations in the city of Graz. After recovery, the sieved and homogenized candidate material was prepared for subsequent analysis, employing acid pressure digestion as well as borate glass fusion decomposition. In the course of the development of natural reference materials for fine ambient particulate matter, batches stemming from different sources have been investigated individually and intercompared, using simultaneous multielement investigation techniques as well as AAS and HPLC. Some inorganic target-compounds including mineralogic major elements and heavy metals trace elements have been determined in addition to a number of well known polycyclic aromatic hydrocarbons.

Finally, results of an intercomparison study between seven environmental analytical laboratories are presented, and fundamental requirements for fine particulate standard reference materials are discussed.

KEYWORDS

Chemical characterization, standardization, fine particulate matter, bulk analysis, trace elements, polycyclic aromatic hydrocarbons, SRM requirements, interlaboratory study.

INTRODUCTION

If environmental analytical chemistry is to be practiced on a routine scale, certain steps have to be taken to insure the comparability of data, one of these provisions is the verification of results with standard-reference materials (Wegscheider, Lorber and Müller, 1978). Observing recent trends in standardization of environmental analytical chemistry, you will find out that there is a significant disagreement concerning the question whether it makes sense or not to invest serious efforts in the development of natural standard reference materials (SRM) for chemical analyses of particulate matter. On one hand, there is definitely a lack of feasibilities for proper calibration of techniques and satisfying verification of results which cannot completely be filled by synthetical standards and reference methods. On the other hand, it seems to be impossible to establish a natural standard material which sufficiently resembles a real "in situ" sample of suspended particles in view of chemical and physical properties.

From the analyst view, particulate matter may be defined as a complex, dynamic system, where over forty elements and well over four hundred different compounds are rather inhomogeneously distributed over a concentration- and particle size range of about seven orders of magnitude. Caused by chemical and physical interactions, this complex matter is subjected to substantial alterations beginning from the time of sampling, up to the final analysis. However, the most drastical change in chemical and physical properties of the collected suspended matter seems to occur during and right after sampling, while in settled particulate matter a large number of components seem to reach a quite stable stationary state (Lorber, 1977).

Recently, an "Urban Particulate Matter Standard Reference Material" which is characterized as to its bulk- and trace element composition has been completed at the National Bureau of Standards, Washington D.C., during the St. Louis Baghouse Project (Gravatt, 1978).

In this paper, our experience in large scale sampling and chemical characterization of settled particulate matter which can be collected in the filters of large air conditioning equipments(like municipal buildings, hospitals, super markets, etc.) are reported.

EXPERIMENTAL

Depending on the type of filter and filter material, exposure time and passed through air volume, up to 50 gram of fine particulate matter can be recovered per square meter of filter surface. For preparation of the raw dust material, the filters are mounted between a plain aluminum sheet and a streched fine nylon network, and the particles are sucked off by a slightly modified vacuum cleaner. Coarse particles can be recovered by

sieving through a 50 µm sieve; and homogenization of the material is preferentially done according to NBS guidlines using a double-coned blender.
For the chemical characterization of this particulate matter, a number of targets-compounds have been selected and determined in our laboratory, using the following analytical methods, Fig. 1.

CHEMICAL CHARACTERIZATION OF URBAN PARTICULATE MATTER

COMPONENTS	METHOD OF ANALYSIS
MAJOR ELEMENTS: Si, K, Ca, Fe	AAS: FLAME AND ELECTROTHERMAL ATOMIZATION HNO_3/HF DECOMPOSITION
TRACE ELEMENTS: Ti, V, Cr, Mn, Ni, Cu, Zn, Pb, Sr	XRF: ENERGY AND WAVELENGTH DISPERSIVE BORATE FUSION
ORGANIC COMPOUNDS: PAH	HPLC: CHEMICALLY MODIFIED COLUMNS ULTRASONIC EXTRACTION PREPARATIVE TLC

Fig. 1. Target compounds and analytical methods used in our laboratory for the chemical characterization of fine particulate material.

AAS with flame and electrothermal atomization as well as energy dispersive and wavelength dispersive XRF are employed for element specific investigations of bulk samples. For AAS measurements, sample preparation is done by HNO_3/HF pressure digestion of 50 milligram of dust sample (Wegscheider, Lorber and Müller, 1978), and in the case of XRF, borate glass fusion decomposition is applied, using 6 gram of $Li_2B_4O_7$ and 500 milligram of dust, according to Lorber and co-workers (1978).As for the organic compounds, a number of selected polycyclic aromatic hydrocarbons (PAH) also have been determined by means of HPLC, after ultrasonic extraction and preparative TLC, Fig. 2.
One of the main problems, connected with the determination of PAH in the investigated samples was to find a proper method for the separation of the organic compounds from the dust particles. This was finally achieved by ultrasonic extraction of portions of up to 200 milligram dust with few milliliters of benzene. Using radioactive labelled (7,10 - ^{14}C) benzo (a) pyrene as a guidance substance, a reproducible recovery of 83 % is obtained after 3 extraction steps of 5 minutes each; and within the investigated range the yield does not depend on the amount of PAH. For the separation of the polyaromatic compounds from the very complex organic fraction, preparative TLC on silica-gel plates was applied, followed by a second ultrasonic extraction step with cyclohexane. For the subsequent analysis by HPLC, a number

of chemically modified supports have been tested, and the separation characteristics have been determined. The best results were found for Nucleosil NO_2 in combination with an isooctane /dichloromethane (9:1)-mixture as mobile phase. Since a fluorescence detector was not available at that time, UV detection at 384 nm was used. At this wavelength, the less carcinogenic PAH show none or little UV response, whereas a number of 5 and 6 membered aromates have absorption coefficients which correspond to detection limits in the low nanogram range.

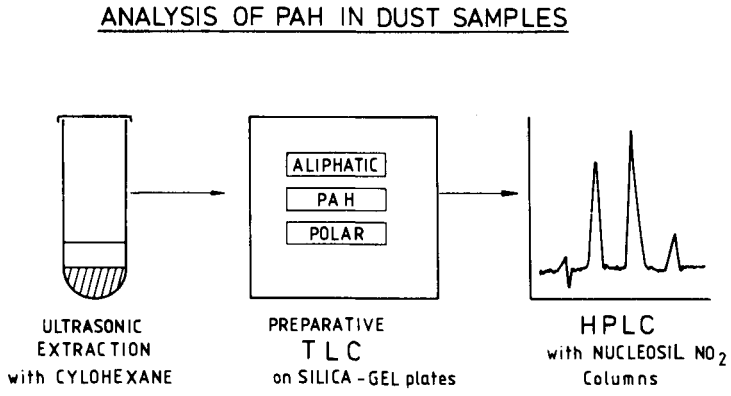

Fig. 2. Analysis scheme for the determination of PAH in dust samples according to Lankmayer and co-worker (1978).

RESULTS AND DISCUSSION

In addition to the chemical characterization of the investigated material, carefully planned experiments have been set up to evaluate the relative size of errors involved in different steps of analysis or attributable to inhomogeneity of the dust samples. In good agreement with the NBS we found, that especially Cu, Zn and Pb seem to be more inhomogeneously distributed than other trace elements (Wegscheider, Lorber and Müller, 1978).
For Pb, the minimum amount for representative samples was estimated experimentally by plotting the standard deviation of the decomposition over the standard deviation of the sample versus the amount of digested sample. The result of Fig. 3 indicates, that at least portions between 50-100 milligram should be taken to reduce the variability of values, and that's about the same outcome which is found by Klockenkämper (1977), using statistical evaluations.
In case of high carbon shares, however, the sample amount for decomposition was limited to 50 milligram, as this was the maximum amount that could be dissolved by the described HNO_3/HF pressure-digestion-technique. But using a mixture of HNO_3/HF /$HClO_4$, sample portions up to 100 milligram could be dissolved.

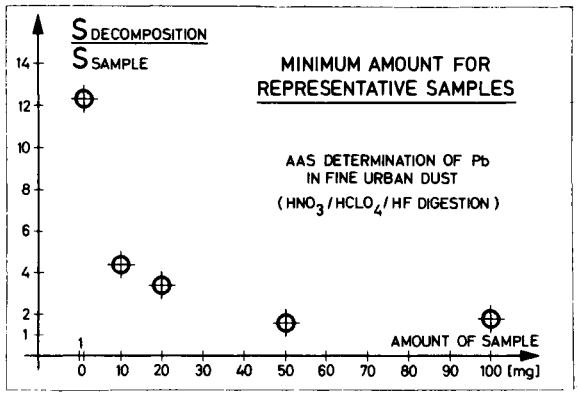

Fig. 3. The standard deviation of the Pb values found in equal amounts of separately digested sample portions over the standard deviation of parallel-measurements within one and the same decomposed sample portion plotted versus the amount of sample.

Investigating different kind of particulate matter we found, that the solubility of the individual compounds generally depends on the origin and nature of the sample material. Trying to isolate the heavy metals from the insoluble matrix, 100 milligram of different dust samples were leached with 20 ml hot HNO_3/HCl. As a result we noticed, that the recovery was significantly non uniform, according to Fig. 4.

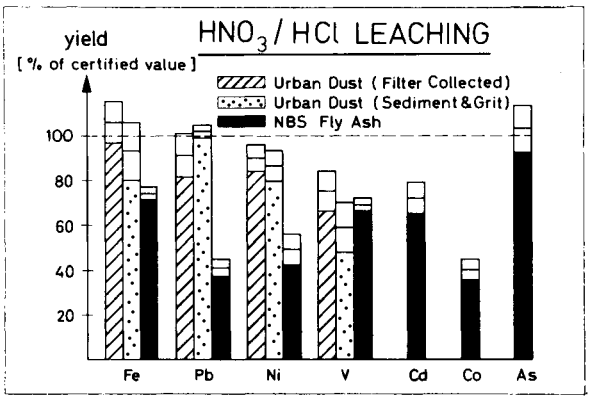

Fig. 4. Yield of the solved components in percent of the certified content, plotted with a confidence limit of 95 %. For Cd, Co and As, there are no reference data available for the ambient dusts.

With the exception of vanadium, the values found for Fly Ash were considerably lower compared to the results found for two different ambient dust samples. This does not surprise, as Fly Ash is a high temperature exposed material, mainly consisting of glass spheres particles. Therefore, using a HCl/HNO_3 leaching procedure for sample preparation instead of total decomposition, a considerable error has to be taken into account. In the course of the chemical characterization of particulate matter for the development of known reference materials, an interlaboratory study was performed, including 7 analytical institutions in 4 different countries (Malissa and co-workers, 1977 and Malissa, 1978).
It can be seen from Fig. 5.,that the standard deviation of the compared results, found for two investigated ambient dusts, is indicating problems with Si, V, Ni and Zn, which can be attributed either to specific errors of analyses or to inhomogeneities of the sample. Probably the first is true for V and Ni, and the latter for Si and Zn.

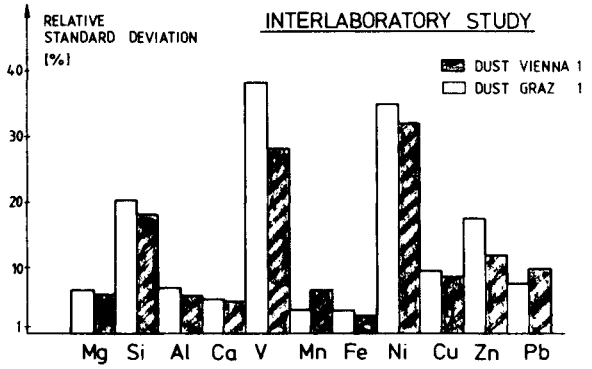

Fig. 5. Relative standard deviation of the compared results of an interlaboratory study.

There is also evidence, that the analyzed dust originating from Vienna is more homogeneous compared to the dust collected in Graz . Except from the major component Fe, the best results we found was for Mn, which is present at the trace amount level. That agrees with the certificate of the NBS Fly Ash, where Mn has the smallest estimated uncertainty of all certified elements.

Concerning the organic compounds adherent to the particulate matter, our investigations have been limited to the determination of some PAH by means of HPLC. A chromatogram of a mixture of PAH standards (A) compared with a chromatogram of a precleaned dust extract (B) is shown in Fig. 6.

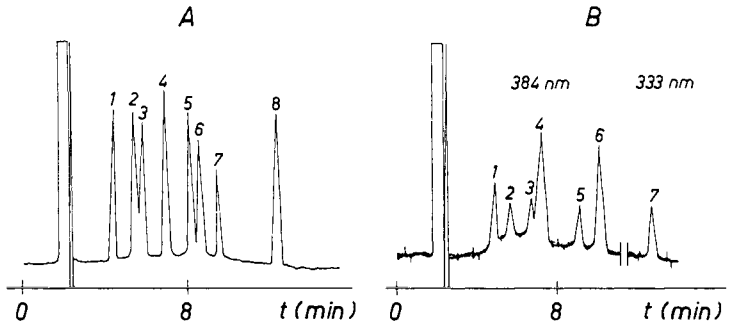

Fig. 6. Separation of PAH on Nucleosil $5NO_2$ column.
A: PAH-standard: 1 = pyrene, 2 = benzo(a) anthracene, 3 = 3-methylcholanthrene, 4 = benzo(a)pyrene, 5 = dibenzo(a,h) anthracene, 6 = indeno (1,2,3-cd)pyrene, 7 = benzo(g,h,i) perylene, 8 = coronene
B: Precleaned dust extract: 1 = not identified, 2 = 3-methylcholanthrene, 3 = benzo(k)fluoranthene,, 4 = benzo(a)pyrene, 5 = indeno(1,2,3-cd)pyrene, 6 = benzo(g,h,i)perylene, 7 = coronene.

Quantitation was achieved by the internal standard addition method, and the difference in peak height was considered to be equivalent to the amount of PAH added to the dust sample. Using UV-detection, the detection limit was about one nanogram.

CONCLUSION

Finally, some fundamental requirements for a fine particulate matter standard material are shown in Fig. 7, and as a conclusion we have to realize that up to now it is not possible to meet all criterias in a satisfying way.

Close Resemblence
Because of alterations, the physical and chemical character of a stored dust standard is somewhat different from the features of a real particulate matter sample.

Nonexpensive Sources
There are sufficient quantities of fine ambient urban dust at hand; and in our opinion it is questionable whether a lot of time, material and work should be spent for a baghouse project (Gravatt, 1978), as the result does not pay for it.

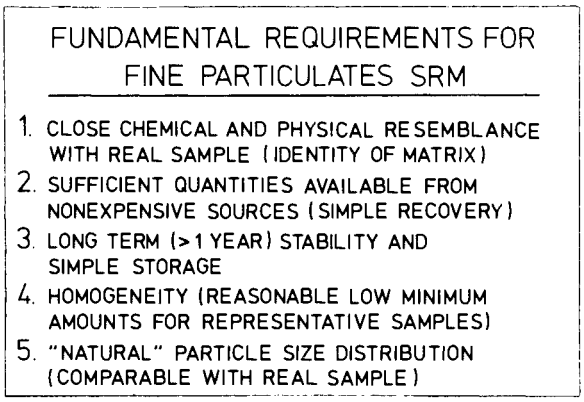

Fig. 7. Fundamental requirements for natural urban particulate matter standard material.

Stability

Until now, long term stability of the dust material has not been rigorously established, but there is evidence, that the stability for metals and PAH is satisfying.

Homogeneity

Dust is an inhomogeneous material by nature, and the minimum amount for a representative sample differs from component to component.

Size Distribution

Because of aggregation, the particle size spectrum of settled dust is quite different from the original distribution of dispersed particulate matter.

Despite of all shortcomings, a natural particulate matter standard material can be a useful help in environmental analysis, especially if destructive relative methods are employed.

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Direct Analysis of Airborne Particles by Glass Matrix X-ray Diffraction

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ABSTRACT

A rapid method has been developed for the analysis of aerosol mineral particles in the size range of 1-10μ following sample collection by filtration of ambient or industrial in-plant air using glass fiber or membrane filters. Sample weights of 20-500μg permit identification and analysis by x-ray diffractometry or infra-red spectroscopy of such aerosols as silica, calcium carbonate and calcium sulfate in actual ambient air samples.

For analysis by x-ray diffractometry (XRD), quantitation is achieved by preparation of standards using known crystalline materials mixed with powdered glass or glass filter fiber as diluent.

Qualitative identification of crystalline aerosols is obtained using the Bragg 2θ diffraction angles to indicate characteristic *d*-spacings. Application of this method has confirmed the presence of such crystalline materials as salt (NaCl), blast furnace slag dust, coke and various process relatable iron oxides.

Membrane filters having a low absorption in the infra-red have been used for collection or for redeposit of mineral dust for comparison to x-ray diffraction methods. Aerosol sulfates have been analyzed using both infra-red spectra and x-ray diffraction.

This method has primary application to the characterization of ambient aerosols, but can easily be applied to the analyses of industrial in-plant suspended or settleable dust.

KEYWORDS

particulate, x-ray diffraction, infra-red, ambient dust, sulfate analysis, slag , aerosols.

INTRODUCTION

The quantitative determination of mineral phases in dust by x-ray diffraction (XRD) has been attempted by numerous investigators since the early work of Clark and Reynolds (1936) on silica particulate. This approach was used successively by Alcocer and colleagues (1969) in the identification and analysis of cement dust in the presence of asphaltic batch mineral aggregate. Later Leroux and Powers (1969) reported the use of membrane filters in collection and identification of silica and several other mineral dusts.

The Wayne County Air Pollution Control laboratory, Warner, Saad and Jackson (1972) first reported the use of amorphous, powdered glass as a diluent matrix for the quantitative determination of settleable particulate pollutants. Most methods which rely on the use of internal standards such as calcium fluoride or kaolin for quantitative determination are limited in scope because of the interference due to the diffraction lines characteristic of the internal standard itself. Application of XRD analysis of proprietary mixtures such as slag dust, sinter fume or graphitic coke required the use of a powder diluent which was as x-ray diffraction transparent, that is, as line free as possible. After experimentation with such materials as hydrocarbon and silicone grease, as well as various mounting oils, powdered, soft glass was chosen as a diluent substrate after repeated scans showed this material to be entirely amorphous.

As a result of these studies, attempts were made to quantitate the composition of total suspended particulate (TSP) directly on high volume air sampling filters since the filter matrix is itself woven glass fiber which would be expected to act as an XRD transparent matrix and diluent.

Initial scans of typical suspended dust samples which represented atmospheric levels of between 100 and 250$\mu g/m^3$ showed little or no discernible x-ray diffraction lines. Since artifically overloaded high volume filter samples could be analyzed with some success following compression of the exposed filter at 24,000 pounds per square inch (1.7×10^8 N m^{-2}), it was assumed that perhaps compressing the exposed filters to a 1 mm wafer caused a concentration of particles which were otherwise spread throughout the 2 mm filter thickness.

In an attempt to produce a maximum concentration effect, 50% portions of several glass fiber filters were subjected to ultrasonic cleaning. Methanol was chosen as a matrix in order to retain the identity of otherwise water-soluble inorganic particulate. Subsequent slurries which contained transparent glass fibers were diluted to a constant weight of 100 mg using powdered glass so that percentage weights of desired contaminants could be based upon a fixed total weight of sample particulate plus glass diluent.

EXPERIMENTAL (X-RAY DIFFRACTION)

The model ADG Beckman Toshiba x-ray diffraction spectrometer was employed together with a Toshiba strip chart recorder which was fitted with a 2θ wavelength event counter. Standard x-ray energy source was a Toshiba iron lamp with K_α 1.92579 Å.

The x-ray tube for the ADG was operated at 15 mA at 40 kVp, together with 1.0-0.3-1.0 slits. A Phillips (Norelco) manganese filter was used to eliminate interference from Fe K_β radiation. The powder mixer-grinder was a Wig-L-Bug model 2A, with stainless steel cylinder and two balls.

Sample Preparation

Success in producing known filter standards was ultimately achieved by preparing a slurry of the desired dust, e.g., slag, silica, limestone etc., together with powdered glass matrix and a known weight of paraffin wax in isooctane. This slurry was slowly suction-filtered through a 47 mm glass fiber air sampling matrix to yield a total weight on the filter corresponding to an average ambient dust sample, i.e., 200-300 mg per 20.3 x 25.4 cm high volume sample surface representing 24-hour exposure. These standard filters were then placed between waxed paper sheets and pressed at 24,000 pounds per square inch (1.7×10^8 N m^{-2}) for 1 minute to allow the paraffin binder to permanently fix the sample to the glass fiber matrix.

Such calibration mixtures produced good x-ray spectral reproducibility which results in the calibration curve, Fig. 1.

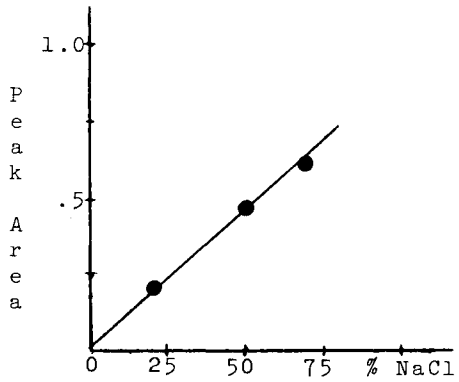

Fig. 1. Calibration of NaCl in powdered glass on glass fiber filters. Sample weight = 10 mg.

Similarly prepared calibration curves for filters containing slag and for calcium carbonate are shown in Fig. 2.

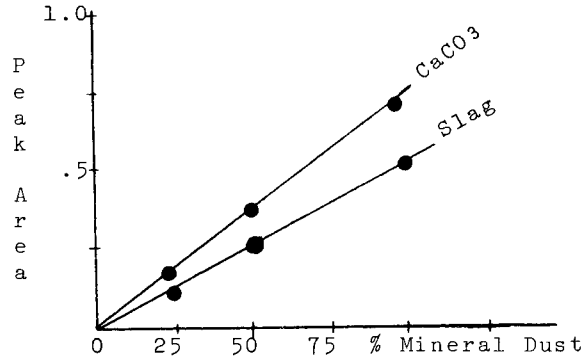

Fig. 2. Calibration of mineral dusts in powdered glass on glass fiber filters. Sample weight = 10 mg.

In the case of silica standards, compression following the deposition of the wax, dust, glass slurry was found to slightly decrease the intensity of the x-ray response as shown in Fig. 3.

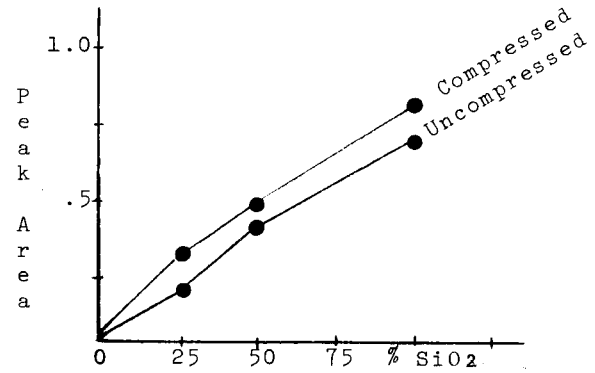

Fig. 3. Comparison of standard filters percentage SiO_2 in powdered glass. Sample weight = 10 mg.

Procedure

Standards are prepared in the following manner. Between 2.5 and 10 mg of the aerosol dust is mixed with an amount of powdered soft glass to yield powder solutions representing 10-90% calibration mixtures. Twenty-five mg of paraffin wax 80°C m.p., is added to the mixture which is then dispersed in 25 ml of isooctane to form a slurry. The slurry is then filtered through a weighed, type A, 47 mm glass fiber filter which exhibits a collecting surface approximately 1/40 that of a standard 20.3 x 25.4 cm glass fiber high volume filter. Following suction filtration, suction air flow is adjusted to 1.2 cfm for 30 minutes which approximates comparable sampling conditions of cfm per cm^2 of filter area. The filter is then reweighed to determine the actual sample weight "collected" and compressed between 5 x 8 cm sheets of waxed paper for 1 minute at 24,000 pounds per square inch for 1 minute. These wafers can then be transferred to a planchette and the x-ray diffraction intensity determined directly by scanning of the appropriate highest intensity 2θ peak for the given material.

Actual exposed high volume filter paper can be analyzed in the same manner by filtration treatment of a 1/40 portion of the filter with 1% paraffin wax in isooctane followed by compression and mounting of the sample on a planchette.

In order to verify the application of this method to the determination of various typical aerosol dusts, control mixtures containing known compositions of aerosol particulate as silica, slag, salt and calcium carbonate were prepared and deposited on 47 mm filters in the same manner as standards.

Comparison of results obtained by x-ray diffraction analysis of this dust mixture is shown in Table 1.

TABLE I Determination of Control Sample Filters

SUBSTANCE IN MIXTURE (1) (2)		PRESENT mg	X-RAY mg	DUST LEVEL $\mu g/m^3$	POWDERED GLASS $\mu g/m^3$
SiO_2	(1)	0.43	0.13	2.6	8
	(2)	0.97	1.0	20	300
$CaCO_3$	(1)	3.0	3.0	60	8
	(2)	1.2	1.5	30	300
Slag	(1)	7.0	5.6	100	8
Dust	(2)	0.82	0.67	34	300
NaCl	(1)	4.1	4.0	80	8
	(2)	0.92	0.80	16	300

Here sample (1) reflects the use of a smaller addition of powdered glass corresponding to a light dust level air sample. Observed silica in sample (1) at a level of 2.6µg/m^3 represents the lower limit of detectability as shown by the incomplete recovery on analysis. Other component substances in the 10-100µg/m^3 range show rather good quantitation to an overall accuracy of ± 11%.

COMPARISON OF X-RAY DIFFRACTION TO INFRA-RED ANALYSIS OF PARTICULATE MATERIAL

Infra-red analysis has also been applied to the analysis of quartz and other hazardous minerals such as asbestos (Bagioni, 1975; Mangi, 1975). A potassium bromide disk containing a sample is used to obtain an infra-red absorption spectra of the sample and the quartz quantified by comparison with prepared standards. Freedman, Toma and Lang (1974) have studied the analysis of quartz on membrane filters having a high infra-red transmittance in the region of the quartz absorption and have compared the method with XRD analysis.

This investigation of the application of infra-red analysis to dust samples was initially undertaken to characterize air suspended particulate in and around an industrial area by sample collection on membrane filters and analysis by XRD and/or infra-red spectroscopy. During these investigations, it was discovered that Nuclepore® membrane filters showed a high transmittance of infra-red radiation in the region of the absorption by sulfate. Precipitation of sulfate as barium sulfate, filtration onto a Nuclepore® filter and measurement of the sulfate absorption at approximately 1060 cm^{-1} provided a simple and sensitive method for sulfate in the range of 20 to 500µg as sulfate ion.

Although several methods have been used for determining molecular sulfate, none are entirely satisfactory when used to determine low levels of sulfate in complex matrices. Analysis using methylene blue, Liang, Sternling and Galloway (1973), turbidimetry, See and Salomon (1974), and titration with barium ion using thorin as an endpoint indicator have been widely applied to environmental samples. Without special care, the turbidimetric method is erratic at low levels. Interfering cation and anions must be removed by ion exchange methods for the barium-thorin titration to be reliable. A further complication in environmental samples results from colored solutions that mask the endpoint of the titration. In a recently published work, Maddalone, Thomas and West (1976) have reported reliable methods for determining low levels of sulfate. Although this latter method, as well as the use of radioisotopes as tracers, is capable of measuring below 1µg of sulfate; special apparatus or extended time for analysis is frequently required.

The following outlines some preliminary work in characterizing minerals and comparing x-ray diffraction to infra-red analysis of air suspended particulate and reports a simple and sensitive method for aerosol sulfates.

EXPERIMENTAL (INFRA-RED)

Mineral standards. Quartz, calcium carbonate, calcium sulfate and dolomite were milled until the average particle size was less than 10μg. Suspensions (50μg/ml) of each of these were prepared in 0.5% surfactant solutions in isopropyl alcohol using an ultrasonic bath. Each suspension was placed in the ultrasonic bath just prior to use. Aliquots were then quickly withdrawn using pipets and dispensed into the filtering apparatus.

Membrane filters. Nuclepore® polycarbonate filters, 25 mm in diameter were used as collection media and as support matrix. A pore size of 5.0μ was used for collecting air suspended particulate. A 0.45μ pore size was used for all suspensions or precipitate filtration.

Procedure - mineral analysis. Standards were prepared by filtering mineral suspensions onto membrane filters. Samples were either collected directly onto membrane filters or removed from collection filters by ultrasonic vibration and redeposited.

Sulfate analysis. Water soluble sulfates were determined by extracting air suspended particulate collected on glass fiber filters. Acid soluble sulfates were determined by extraction with 1:1 nitric acid. The solution was evaporated to dryness and the residue taken up in a minimum of 6NHCl and diluted with distilled water. Aliquots of the sample solutions were removed and filtered through 0.45μ filters. The pH was adjusted with dilute NaOH or HCl, and the sulfate precipitated with 5% solutions of barium chloride, and filtered onto membrane filters.

Infra-red spectra were obtained using a blank filter in the reference beam. The x-ray diffraction intensities were measured by mounting the filter on an aluminum holder and scanning over the angles of interest. Quartz standards in epoxy or silicone polymers were used to correct for long term drifts in x-ray intensity.

Various minerals such as calcium sulfate and dolomite were deposited on Nuclepore® filters and irradiated at their absolute maximum using infra-red radiation. Results of these analyses are shown in Figs. 4 and 5.

RESULTS AND DISCUSSION

A quantitative survey method has been established for the determination of mineral dusts in ambient air or industrial hygiene atmospheres. Dust samples may be collected on chemically inert glass fiber filters and may be x-ray diffraction scanned directly after treatment with a dilute solution of paraffin wax in isooctane and compression in a laboratory press.

Standards are prepared by filtering suspensions containing known weights of powdered mineral dusts, glass particulate, and wax binder in isooctane through glass fiber filters and compressing the filter before mounting on a glass planchette for x-ray scanning.

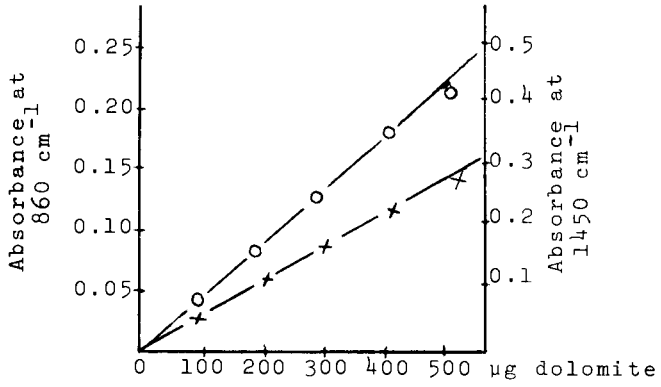

Fig. 4. Reproducibility of dolomite standards at 2 major infra-red absorption maxima.

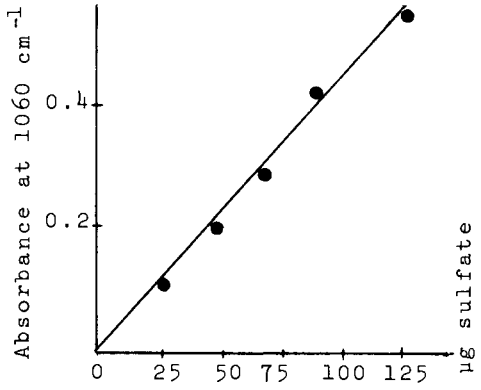

Fig. 5. Reproducibility of calcium sulfate redeposited on Nuclepore® filters using infra-red radiation.

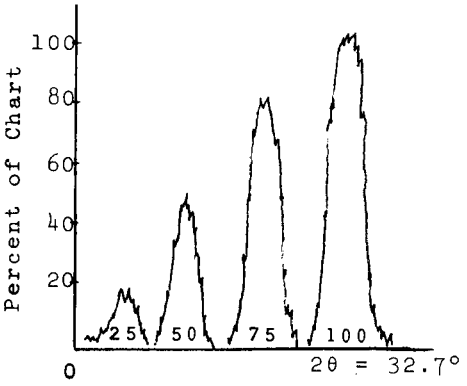

Fig. 6. Variation peak area showing distortion of peak at low sulfate levels at a single angle at 2X attenuation.

TABLE 2 Comparison of Infra-red and X-Ray Diffraction Analyses of Ambient Dust Samples Containing Particulate Sulfate

Date (1977)	Infra-red	X-Ray Diffraction*
June 7	14.7	15.8
10	11.7	13.2
12	4.0	3.5
14	8.5	12.5
16	22.0	28.0
22	12.8	11.5
24	27.1	37.0
27	20.8	22.4
30	17.6	19.9
July 10	13.7	15.0

*Average of Peak Areas at 32.7°2θ and 31.0° 2θ.

Mineral dusts such as slag, silica, limestone, and salt have been analyzed at levels between 10 and 100μg/m^3 to an accuracy of ± 10%. As a result, the method can be expected to yield good survey accuracy at well below the threshold limit values (TLV's) of most mineral dust and at levels within the ordinary sampling range of urban ambient air.

Sulfate standards precipitated as barium sulfate and filtered onto Nuclepore® filters results in a linear absorption of infra-red radiation at approximately 1060 cm^{-1}. The method was capable of detecting less than 10μg of sulfate ion and gave a reproducibility of approximately 2% at the 100μg level. The method was not affected (within experimental error) by variation in acid concentration from pH 2 to 6.5 during the precipitation of barium sulfate, which is in contrast to the effects reported in the turbidimetric analysis of sulfate, See and Salomon (1974). X-ray analysis of the precipitated barium or calcium sulfate generally agreed with the infra-red analysis, Table 2, where samples represent alternate day ambient air sampling near a steel mill and x-ray results were obtained by averaging peak areas at 32.7°2θ and 31.0°2θ to minimize the effect of interferences (d = 3.44 and 3.62, respectively). Here it was discovered that at least two crystalline forms occurred during the precipitation of barium sulfate. Variation in the amount of each of these forms caused variations in the diffraction intensity and configuration of the peak heights used in the analysis as shown in Fig. 6.

Increased sensitivity of the infra-red method should be possible if the precipitated barium sulfate were filtered onto a smaller area. At least a fourfold increase in sensitivity appears possible and would result in reliable analysis of sulfate below 10μg. The XRD method proved more sensitive than infra-red absorption for the minerals studies; however, an increase in sensitivity is possible for the infra-red method by also filtering onto smaller areas.

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Ion-selective Electrodes in Environmental and Toxicological Analysis

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ABSTRACT

Ion-selective electrodes have been widely employed for air and stack gases, water supplies and sea water, rocks and soils, vegetation, etc. Most applications have involved nitrate, fluoride and chloride electrodes, although most other types have also been employed. Some applications have involved gas sensors, such as in sulphur dioxide determinations in flue gases and wines, ammoniacal nitrogen and organic carbon in water.

Environmental analysis with ion-selective electrodes extends into areas of toxicological concern and may include areas such as nitrate levels in forage and vegetable crops, nitrogen species in air and combustion emission, cyanide contents of glycoside materials like sorghum, linseed and almonds, and the effect on man, animals and plants of fluoride and other emissions from industrial complexes.

Toxicological concern may also not be so obviously connected with the environment and may include such matters as the constituents of cigarette smoke and atmospheric particulates, the use of brominated vegetable oils in the soft drink industry, and the metabolic products resulting from fluorine-containing inhalent anaesthetics.

The advantages, scope and limitations of ion-selective electrodes for the above kind of applications are discussed, with special reference to nitrate and fluoride ion-selective electrodes in the analysis of various sample types, including rocks and soils, plant extracts, water air and combustion emissions, and anaesthetic metabolism studies. Attention is given to sample pre-treatment and measurement precautions, with access to many relevant references.

KEYWORDS

Air pollutant analysis; anaesthetic (fluorinated) metabolism; combustion emission analysis; environmental analysis with ion-

selective electrodes; ion-selective electrodes in environmental and toxicological analysis; plant extract analysis; soil and rock extract analysis; toxicological analysis with ion-selective electrodes; water analysis.

INTRODUCTION

In the narrowest sense chemistry of the environment is concerned with air, water, rocks, soils and vegetation, but consideration of the effects of erosion, emissions and effluents greatly widens the scope. Further extensions arise through toxicological concern, not just from the effect of polluting effluents but also from less obvious sources, like excessive nitrate in forage, the presence of cyanide in glycoside materials of plant origin, the use of brominated vegetable oils in the soft drink industry, carcinogenic constituents of cigarette smoke, metabolic products resulting from fluorine-containing inhalent anaesthetics, etc.

Ion-selective electrodes are widely applicable for monitoring ions in these areas. Most applications have involved nitrate, fluoride and chloride electrodes although many others have also been employed including the gas sensors used for sulphur dioxide in flue gases and wines, ammoniacal nitrogen, organic carbon in water etc.

It is convenient to deal with this wide ranging subject matter under three main headings, namely, general principles of ion-selective electrodes, measurement methods and illustrative applications in environmental and toxicological analysis.

PRINCIPLES OF ION-SELECTIVE ELECTRODES

Design

Ion-selective electrodes (Moody and Thomas, 1971a, 1973, 1977a) are a group of selective potentiometric ion-sensitive devices of which the hydrogen ion-sensitive glass electrode is the most important member. Like the glass electrode in which a glass membrane separates two aqueous phases, ion-selective electrodes in principle have a membrane separating the inner reference solution from the test solution. This membrane may be
(a) a solid-state crystal, as for example, lanthanum fluoride in the fluoride ion-selective electrode;
(b) a precipitate impregnated polymer membrane, for example, a silver halide precipitate in silicone rubber;
(c) a liquid ion-exchanger, for example, a tetraalkylammonium nitrate sensor with n-octyl 2-nitrophenyl ether or other solvent mediator and supported on a millipore filter, porous ceramic disc, etc., or trapped in a PVC matrix membrane;
or (d) a neutral carrier sensor, for example, valinomycin of the potassium ion-selective electrode used in conjunction with a solvent mediator and set up in a membrane as for the liquid ion-exchanger systems.

In many present-day ion-selective electrodes the inner aqueous reference solution is dispensed with and there is direct contact

of the electrical conductor to the membrane, for example, to the crystal in solid-state electrodes, and a wire coated with liquid ion-exchanger in a PVC solution for coated-wire electrodes.

Ion-selective electrodes come in many forms of mechanical design and may be scaled down for micro work. They may be modified for such specialised applications as gas sensors (Ross, Riseman and Krueger, 1973) and enzyme/substrate sensors (Montalvo and Guilbault, 1969; Moody and Thomas, 1975). In the former case, a gas permeable membrane (Ross, Riseman and Krueger, 1973) or an air-gap (Růžička and Hansen, 1974a and b) separates the sample solution from a thin film of an intermediate solution which is either held between the gas membrane and the ion-sensing membrane of the electrode, or placed on the surface of the electrode using a wetting agent as in the air-gap electrode. The intermediate solution interacts with the gaseous species in such a way as to produce a change in a measured value, such as pH of the intermediate solution. This change is then sensed by the ion-selective electrode and is proportional to the partial pressure of the gaseous species in the sample.

Enzyme-substrate electrodes are sensors in which an ion-selective electrode is covered with a coating containing an enzyme which causes the reaction of an organic or inorganic substance (the substrate) to produce a species to which the electrode responds. Alternatively, the sensing electrode membrane could be covered with a layer of substrate which reacts with the enzyme to be assayed.

Principles of Use

In use, and when operating properly the ion-selective electrode potential, E, with respect to a reference electrode, such as a saturated calomel electrode, is given by (Moody and Thomas, 1971, 1973, 1977a and b)

$$E = \text{constant} + S[\log a_A + k_{AB}^{pot}(a_B)^{z_A/z_B}] \qquad (1)$$

Thus, the ion-selective electrode responds selectively to an ion, A, of charge, z_A, and activity, a_A, in the presence of an interfering ion, B, of charge, z_B, and activity, a_B; k_{AB}^{pot} is the selectivity coefficient. The constant term incorporates various junction potentials (except for the membrane-test solution boundary) as well as the standard potential characteristic of the ion-selective electrode. Normally, the calibration slope, S, approaches the Nernstian value of $2.303\ RT/z_AF$, and this holds over a wide p(Ion) range.

Selectivity

Equation (1) allows for the fact that specificity is rather exceptional for ion-selective electrodes and further terms are required where competition for electrode response is exerted by species additional to A and B. A common interference effect is the fall-off in linear calibration restricting the scope of the

electrode to a lower p(Ion) range. However, in addition to competitive response there can be other kinds of interference such as those that occur by the presence of complexing species reducing the true levels of the primary ions, A.

A common method of obtaining the selectivity coefficient, k_{AB}^{pot}, is to measure the emf response of solutions containing a fixed amount of interferent with varying activities of the primary ion, A, for which the electrode is designed (IUPAC, 1976; Moody and Thomas, 1971a and b, 1973, 1977a and b). This is known as the mixed solution method with fixed amount of interferent and k_{AB}^{pot} is calculated from

$$k_{AB}^{pot} = \frac{a_A}{(a_B)^{z_A/z_B}} \qquad (2)$$

a_A and a_B appertain to the intercept of that part of the calibration curve (of near zero slope) corresponding to complete interference by the interferent, B, with that of slope corresponding to more or less unfettered response by the primary ion A. The level of B corresponding to k_{AB}^{pot} should always be quoted for it is then a simple matter to calculate by equation (2) the useful limit of the electrode for ion A.

An alternative mixed-solution method (IUPAC, 1976; Moody and Thomas, 1971a and b, 1973, 1977a and b) for expressing selectivity involves varying the interferent activity, a_B, at constant primary ion activity, a_A. This method is normally used for expressing the pH range over which ion-selective electrodes are useful.

Interference Elimination

The use of ion-selective electrodes in environmental and toxicological applications can be complicated owing to the variable nature of samples. There may be variable levels of interferents, different levels of complexing equilibria, poisoning effects of colloidal electrolytes, etc. Such complications hinder routine use and there are also factors concerning variations in ionic strength. Clearly the devices demand a higher level of technical competence than, say, the glass electrode for pH measurements but this can be compensated by rapid access to data on ionic levels so frequently the real variables of interest. For example, there is much greater concern over free cyanide than complexed cyanide.

Interference can frequently be overcome, for example, low pH buffer systems containing silver sulphate and aluminium sulphate in nitrate determinations, maintain the equilibrium bicarbonate low, keep the water-extractable organic acids undissociated, remove chloride as silver chloride and complex anions of organic acids with aluminium (Milham and co-workers,1970; Sweetsur and Wilson,1975).

Because ion-selective electrodes sense activity rather than concentration, they ought to be used under conditions of constant ionic strength. This can be done by mimicking the ionic strength,

but this can be difficult because of frequent considerable overall variations in composition that can occur in samples. A more practical alternative is to add an "ionic strength adjuster" to both sample and calibrating solutions in order to achieve a common ionic strength level, to release the ion of interest from complexes and to buffer samples to within regions of minimal pH interference.

Although virtually any ionic substance may function as an ionic strength adjuster, it must not complex with the primary ion, and k_{AB}^{pot} must be negligible - B in this case being the ionic strength adjuster constituent. Several systems have been devised and a well-known one is TISAB (Total Ionic Strength Adjustment Buffer) used in fluoride determinations (Frant and Ross, 1968).

In exceptional circumstances, for example with high chloride levels in soil extracts, it may be necessary to remove the interfering species from the test situation by precipitation, ion-exchange resins or otherwise.

MEASUREMENT METHODS

The important and widely used measurement methods with ion-selective electrodes include direct measurement, standard addition and subtraction methods, Gran's plots and potentiometric titrimetry with some attention to differential and null-point potentiometry (Moody and Thomas, 1971a, 1973, 1977a and b; Thomas, 1978).

Direct Measurement

Direct measurements are based on the linear Nernstian or near-Nernstian logarithmic relation between emf and ionic activity. This, of course, requires attention to calibration, including calibration checks with standards interspersed between samples, and regard to competing response by interfering species.

It is important to realise that high precision is rarely achieved in direct measurement, for quite apart from variation due to electrode drift and problems of the reference electrode, general laboratory conditions are such that readings to within $\pm$ 0.1 mV are difficult. This in itself leads to an error of $\pm$ 0.4 per cent in a_A under Nernstian conditions for an univalent ion and double for a bivalent ion.

Precision voltmeters, reading to $\pm$ 0.1 mV or better are necessary for the best work and a change of just 8.9 mV in emf for a doubling or halving of the activity of a bivalent ion emphasises this need.

Continuous Monitoring and Flow Systems

The principles of direct measurement are readily adapted for the automatic or semi-automatic analysers based on continuous flow systems. These have advantages by the standardised way in which the sample is presented to the ion-selective electrode sensor.

This applies also to the more recently developed flow-injection systems (Růžička and Hansen, 1975,1977,1978).

The characteristic fast time responses (1 or 2 s or less) of ion-selective electrodes (Moody and Thomas, 1974) under the dynamic conditions of continuous flow systems are relevant to environmental surveillance. Furthermore, sample streams can be pretreated in order to overcome interferences and problems of variable ionic strength, or to provide the correct pH conditions.

Continuous methods have also been developed for "scrubbing" gases with suitable reagents, and arrangements made for measuring the quantities of gas and reagent with flow transmitters in order to appropriately quantify the ion-selective measurement of the soluble component in a flow-through cell (Thomas, 1978).

Direct immersion of electrodes in an effluent stream has the risk of not giving sufficient accuracy and precision and apart from the need for sample pretreatment, there are limitations due to variations in temperature and the tendency for sensor surfaces to become coated with particulate matter, oil or biological growths. Thus, it is best to bleed off the sample from the source and pump through a filter into a thermostatted monitor associated with the addition of any pretreatment agent that may be required. In these the sample stream may be switched off at intervals for checks and replaced by a stream of standard for calibration and stability checks, when it is recommended (Bailey, 1976) that the standard solution should be at approximately the middle of the range of the instrument. This ensures analysis to a possible $\pm$ 5 per cent degree of precision.

Standard Addition and Subtraction Methods

Known addition, known subtraction and Gran's plot methods are ways of using ion-selective methods that complement the direct method (Moody and Thomas, 1973,1977a and b; Thomas, 1978). In the standard addition to sample method, the emf, E_o, of the ion-selective electrode with respect to a suitable reference electrode is measured for the sample of volume, V_o, and total concentration, C_o, of the sought species:

$$E_o = \text{constant} + S \log x_o f_o C_o \qquad (3)$$

where f_o is the activity coefficient and x_o the fraction of uncomplexed ions. A new emf, E_a, is measured after addition of a small volume, V_a, of a standard solution (concentration C_s) of ions of the species sought, where $C_s \sim 100\ C_o$

$$E_a = \text{constant} + S \log x_a f_a \frac{(V_o C_o + V_a C_s)}{(V_o + V_a)} \qquad (4)$$

where f_a and x_a correspond to the new activity coefficient and fraction of free ions, respectively. An essential assumption is $x_o = x_a$ and $f_o = f_a$. Hence the difference, ΔE, between E_a and E_o can be expressed and C_o resolved

$$C_o = \frac{C_s}{10^{\Delta E/S}(1 + V_o/V_a) - V_o/V_a} \quad (5)$$

or for no allowance for volume change, that is, V_a small in relation to V_o

$$C_o = \frac{C_s V_a}{(10^{\Delta E/S} - 1)V_o} \quad (6)$$

For analate addition purposes, that is, sample addition to standard, the corresponding version of Equation (5) is

$$C_o = C_s[10^{\Delta E/S}.(1 + V_s/V_{oa}) - V_s/V_{oa}] \quad (7)$$

where V_{oa} is the volume of sample solution added.

The known subtraction method depends on lowering the level of uncomplexed ions by adding a complexing agent when the structures of the equations depend on the nature of complexation (or precipitation), that is, 1:1 or 1:n.

An interesting variation of the standard addition method depends on a modification of the method described by Gran for presenting potentiometric titration data in linear form using a semi-antilog plot. The principle is apparent in Equation (4) above for which the antilog version is

$$(V_o + V_a)\ 10^{E_a/S} = x_a f_a\ 10^{\text{constant}/S}(V_o C_o + V_a C_s) \quad (8)$$

A plot of the left-hand side of Equation (8) as ordinate versus V_a as abcissa gives a straight line to intercept the abcissa for a V_e value where

$$C_o V_o = - C_s V_e \quad (9)$$

and from which the concentration of the ion under test, C_o, can be easily calculated.

Computation of the left-hand side of Equation (8) is avoided by using semi-antilog Gran's Plot Paper supplied by Orion Research Incorporated which has been corrected for limited volume changes. Alternatively, a ruler can be prepared to take in the left-hand side of Equation (8) for the direct plotting on ordinary graph paper of ordinate data from E_a readings.

Potentiometric Titrations

At higher concentrations, potentiometric titrations are superior to direct potentiometry. There is also the added advantage of the possibility of using the ion to which the indicator electrode

is selectively sensitive as the titrant in determining species for which ion-selective electrodes are not even available. For example, a barium ion-selective electrode can be used to monitor the titration of sulphate with barium chloride or perchlorate (Jaber, Moody and Thomas, 1976).

Applications in titrations need to have regard to distortions that can occur in potentiometric titrations curves, and of course, the effect of interferents need to be borne in mind.

Differential and Null-Point Potentiometry

Differential and null-point potentiometry may be employed with ion-selective electrodes. The former is a concentration cell technique involving the use of a matched pair of electrodes whose liquid junction potentials become negligible when a sufficiently large excess of an inert electrolyte is used. The unknown solution is placed in one half-cell and a standard solution in the other. The potential difference is related to the ion concentration by a calibration curve.

The precision of the differential method can be improved by using null-point potentiometry. The ion concentration is measured, not from a single emf reading, but by adjusting the composition of one of the half-cell solutions to match the other until a potential (the null, or bias potential) is obtained that corresponds to that prevailing when both half-cells contain an identical solution.

ILLUSTRATIVE APPLICATIONS

Rather than provide a catalogue of all the applications of ion-selective electrodes for various sample types, it is more appropriate here to discuss some typical applications of a limited range of electrodes, measuring situations, and of the main features of the data obtained.

Nitrate

The nitrate ion-selective electrode (Ross,1969; Danesi,Scibona and Scuppa,1971; Davies,Moody and Thomas,1972; Nielsen and Hansen, 1976) has been widely used for environmental and toxicological-type surveillances. These include plant and soil extracts for nitrate nitrogen, and cigarette smoke and the atmosphere for oxides of nitrogen. This electrode can therefore be used to illustrate the handling of several sample types.

Rocks and soils. The electrode is as reliable as established colorimetric methods for determining nitrate-nitrogen in rocks and soils, but chloride and nitrite can interfere unless removed. For nitrate-nitrogen levels of below about 1 mg dm^{-3} in the extract, a preconcentration stage is recommended.

A wide-ranging study (Øien and Selmer-Olsen,1969) of the scope of the Orion 92-07 nitrate ion-selective electrode for nitrate determination in soil extracts has shown that there is no significant difference between the nitrate levels of soil suspensions

in the extracting media or their filtrates. This is of importance since filtration of clay or peat soils can be tedious, especially in field conditions. Water suffices as an extractant but then the analyses need to be immediate in order to avoid biological losses, although these can be prevented by extracting with 0.01M copper sulphate and almost all nitrate-nitrogen is extracted in two minutes (Øien and Selmer-Olsen,1969).

Analytical results for the nitrate content in 0.01M copper sulphate soil extracts obtained by the nitrate ion-selective electrode compare with those of the colorimetric xylenol method and with colorimetric AutoAnalyser determinations on 2M potassium chloride extracts. Examples are shown in Table 1.

TABLE 1 Nitrate-Nitrogen Content of Soil Extracts (Øien and Selmer-Olsen,1969)

Data are expressed as mg nitrate nitrogen per dm^3 of extracting solution

Soil type	Extractant/Method		
	Copper sulphate 0.01M		Potassium chloride (2M)
	Electrode	Xylenol	AutoAnalyser[a]
High organic sand	21.0	21.6	21.0
Sandy peat	13.1	14.2	14.0
Peat soil	1.6	1.5	1.5
Peat soil	14.6	14.7	15.0
Peat soil	38.9	38.0	39.3
Peat soil	48.0	50.0	49.8

a Using sulphanilamide and N-1-naphthylethylenediamine reagents

However, t-tests show a small, but significant difference between the extracting agents for low nitrate with 2M potassium chloride being slightly more efficient than copper sulphate. Chloride at such a high level cannot be used as an extractant for determinations by electrode because of interference problems. There may also be certain other interferences introduced from soil into the extracts and it is for this reason that the buffer system, already mentioned above was introduced for making ion-selective electrode measurements (Milham and co-workers,1970). A later study on grass has shown that interfering ions are not completely removed by this buffer and a stronger one made up of 0.025M aluminium sulphate, 0.025M silver sulphate, 0.050 boric acid, 0.050M sulphamic acid and sufficient 0.1M sulphuric acid to adjust the pH to 3.0 was therefore recommended (Sweetsur and Wilson,1975).

An automatic apparatus for extracting with water (10 g soil + 25 cm^3 water) and analysing for nitrate (by automatic insertion of a Corning nitrate ion-selective electrode with reference electrode) caters for batches of up to 60 soil samples (Goodman, 1976). The emf output is amplified and recorded on a chart recorder. 94-95 per cent recovery of added nitrate is claimed with an estimated 1.9 per cent standard error for a single measurement. The apparatus can be used for pH as an alternative to nitrate and at

the time of submission for publication had been used for more than 4000 soil samples (more than 6000 samples by September 1978). During appraisal, it was found that an Orion 97-07 nitrate ion-selective electrode was inferior to the Corning electrode and gave only 88 per cent of the values obtained by steam distillation with titanium(III) sulphate (Goodman,1976). Nevertheless, Bound (1977) used the ion-exchanger of the Orion 97-07 electrode in a PVC matrix membrane for measuring nitrate levels of soil pastes with saturated calcium sulphate solution (in order to make the ionic strength constant). The following regression equation was computed for 60 soils, the nitrate data being expressed in mg N dm^{-3} soil solution at saturation.

$$(\text{1M-KCl extraction value by AutoAnalyser}) = 2.84 + 0.45\ (\pm 0.03)(\text{electrode value}) \quad (10)$$

$$r = 0.863$$

Large quantities of nitrogen fertilisers will result in toxic levels of nitrate in forage and vegetable crops since ammoniacal-based fertilisers are rapidly oxidised by microbiological action to nitrate and soon becomes the major nutrient nitrogen species in the soil. Nitrate absorption in crops may then exceed its natural reduction and assimilation.

Generally, nitrate exerts no phytotoxic effects (Moody and Thomas, 1977b) but nitrate-rich fodder, hay and silage have poisoned grazing animals. However, there are no authenticated cases of human adults being poisoned after consuming high nitrate level vegetable materials, although very young babies have suffered illness following spinach feeds of high nitrate content.

Another detrimental effect is that about 35 ppm of nitrate-nitrogen in canned vegetables is sufficient to completely de-tin the internal surface of the container in about six months. Further problems may arise by the microbiological reduction of nitrate to nitrite in stored vegetables or in the gastrointestinal tract since interaction with haemoglobin forms methaemoglobin nitrite - the toxic factor in nitrate poisoning.

Plant tissues. Analysis of plant tissue for nitrate is frequently used to supplement soil tests in order to guide both fertilisation and harvesting planning schedules and although this cannot be regarded as being strictly within the scope of the title, it is interesting to look briefly at the methods employed.

Paul and Carlson (1969) extracted nitrate from dried, ground plant material (~400 mg) by shaking with water (50 cm^3) for 10 to 15 minutes in an Erlenmeyer flask. For accurate work prior removal of chloride is recommended when tissues contain <500 ppm nitrate-nitrogen and >2 per cent chloride. This is readily achieved by adding Dowex 50-X8 (Ag^+ form) ion-exchanger resin rather than silver sulphate with the added advantage that the anion, being part of the resin, is retained after filtration whereas sulphate could interfere while the excess silver ions would react with the calomel reference electrode. Dowex 50-X8 (Al^{3+} form) resin is

also included to remove interference from bicarbonate and unspecified organic acids. Here again, filtering, ostensibly to offset possible fouling of the electrode by plant colloids, did not yield any different results with the Orion 92-07 ion-selective electrode from those observed before filtration. Also, nitrate assays of sixteen different samples by the potentiometric and phenoldisulphonic acid methods agreed closely (Paul and Carlson, 1969).

Close agreement was also obtained by Milham and co-workers (1970) for direct potentiometry with an Orion 92-07 nitrate ion-selective electrode and a modified Devarda's alloy method of the nitrate-nitrogen contents of six different plant species, namely, cotton petiole and of oat sorghum, maize, wheat and tomato leaves.

Air and combustion emissions. Interesting applications of the nitrate ion-selective electrode extend to nitrogen species in air and combustion emissions including cigarette smoke. The analysis of these samples can also use the services of other sensors of nitrogen species. In this respect it must be remembered that oxides of nitrogen, ammonia, ammonium, nitrate, nitrite and diverse nitrogen organic matter are important parameters in the nitrogen cycle.

The Orion NO_x gas electrode measures nitrous acid, or rather it monitors an equimolar gaseous NO-NO_2 mixture in equilibrium with aqueous nitrous acid, or equally well the nitrite ion itself which is conveniently converted to nitrous acid merely by acidification (Orion Research Inc.,1973). Alternatively, these gaseous nitrogen species can be variously converted to nitrate and so measured with a nitrate electrode. Also nitrate can in turn be reduced either to nitrite or ammonia and then measured with the appropriate gas-sensing electrode (Moody and Thomas, 1978).

Over 60 per cent of gaseous NO_x (largely nitric oxide) emission in the USA comes from stationary combustion sources varying from 20 ppm for small gas-fired boilers to 1400 ppm for coal-fired power plants. Automobile exhausts also contribute to atmospheric NO_x levels.

Generally NO_x levels have been estimated as nitrate with a nitrate ion-selective electrode following conversion to nitrate, rather than as NO_x with a NO_x gas electrode. However, quantitative conversion to nitrate irrespective of origin is not straightforward and various methods have been used.

DiMartini (1970) determined NO_x with a nitrate electrode following gas-phase ozonisation and aqueous absorption-hydrolysis of dinitrogen pentoxide, and also any remaining dinitrogen tetroxide, to nitrate in 10^{-4}M sodium nitrate.

$$NO + O_3 \longrightarrow NO_2 + O_2 \qquad (a)$$

$$2NO_2 + O_3 \longrightarrow N_2O_5 + O_2 \qquad (b)$$

$$N_2O_5 + H_2O \longrightarrow 2HNO_3 \qquad (c)$$

$$3NO_2 + H_2O \longrightarrow 2NO_3^- + 2H^+ + NO \quad (d)$$

Spiked hydrolysates are then passed through a flow-cell coupled to a nitrate-reference electrode pair. Air levels as low as 10^{-8}M nitrate corresponding to operative tunnel automobile traffic conditions can be so detected (DiMartini, 1970).

Kneebone and Freiser (1973) similarly determined NO_x levels in ambient air following conversion to nitrate with 2 per cent hydrogen peroxide. A 40-fold excess of sulphur dioxide and trioxide could be tolerated, but excess peroxide had first to be destroyed with finely divided manganese dioxide. The "nitrate" levels in down-town Tucson air by the colorimetric xylenol and nitrate electrode methods agreed to within 1.5 to 2 per cent relative standard deviations. The difference between figures for two days, namely, 119 and 216 $\mu g\ m^{-3}$ of NO_x is attributed to traffic flow variations although changes in air movement can also have this effect.

Oxides of nitrogen in combustion gases have been similarly analysed following oxidative absorption on lead dioxide specially prepared from old anode battery plates (Driscoll and co-workers,1972):

$$NO + PbO_2 \longrightarrow NO_2 + PbO \quad (e)$$

$$2NO_2 + PbO_2 \longrightarrow Pb(NO3)_2 \quad (f)$$

Unlike chloride, fluoride or sulphur dioxide, carbon dioxide does not react with the lead dioxide. Control experiments with fixed levels of nitric oxide and various other gases indicated the amount of interference to be expected and did not give cause for concern (Table 2).

TABLE 2 Nitric Oxide Recoveries from Known Mixtures of Gases (Driscoll and co-workers,1972)

Actual NO = 211 ppm

Foreign gas(ppm)		Nitric Oxide found (ppm)	
		Nitrate electrode	Phenoldisulphonic acid
2220	SO_2	223 → 227	187 → 193
1080	SO_2	211 → 222	211 → 217
2400	HCl	192 → 209	182 → 212
1500	HCl	211 → 218	187 → 196
14000	CO_2	214 → 219	201 → 222
1700	HF/SiF_4	197 → 203	224 → 244
		$\bar{X}$ 212 (sd = 9.6)	$\bar{X}$ 210 (sd = 17.5)

Airborne particulates and cigarette smoke. The nitrate ion-selective electrode has been introduced into continuous-flow systems for monitoring atmospheric particulate nitrate (Driscoll and Forney,1974). In a more recent flow-through electrode unit a fixed volume of de-ionised water is recirculated through an aerosol impaction device (ERC LEAP sampler, Model 3440) in which the nitrate particulate is collected and dissolved and returned to the electrode housing for nitrate ion measurement (Forney and McCoy, 1975).

The determination of ammonium ion in airborne particulates is important since atmospheric ammonium sulphate relates to health, corrosion and visibility. Thus, ammonium ions have been measured on aqueous extracts of samples collected on glass fibres from high volume air samples (2000 m^3) down to 0.03 $\mu g\ m^{-3}$ with a Beckman ammonium ion-selective glass electrode and an Orion ammonia gas-sensing electrode (Eagon and DuBois,1974). Each of these possess advantages. The Beckman electrode is more accurate at lower levels and the Orion electrode at higher levels when interfering cations become more effective in their significance for the Beckman electrode.

Aqueous solutions with an inbuilt natural pH <7.5 permit assay as NH_4^+ ions with the Beckman electrode, while pH adjustment to >11 with sodium hydroxide in order to convert to NH_3 species permits a check assay with the gas electrode. The mean Orion value at 1.7 ppm (mean deviation = 0.19 ppm) compares with 1.58 ppm (mean deviation = 0.28 ppm) for the Beckman electrode and also includes sampling errors (Eagon and DuBois,1974). Surveys for Ottawa City during 1972-73 and Montreal in 1969-70 established that only very small amounts of sulphate can be present as $(NH_4)_2SO_4$ and refute the concept (Junge,1963) that all of the NH_4^+ ions are associated with sulphate. The main products of sulphur dioxide combustion can also exist as calcium sulphate or sulphuric acid. .

Sulphur dioxide levels of flue gases can exceed 5000 ppm. This can be estimated with the sulphur dioxide gas electrode or by oxidation to sulphate after collection. The sulphate can then be determined by potentiometric titration with lead perchlorate and a lead ion-selective indicator electrode (Driscoll, Mahoney and Young,1973) although barium chloride or barium perchlorate may also be used with a barium ion-selective electrode (Jaber, Moody and Thomas,1976). The lead perchlorate method has been tried and gives results comparable with the barium-thorin and barium chloranilate colorimetric methods for flue sulphur dioxide from plants of sulphuric acid, lead smelting, iron and steel, a Kraft mill and a coal-fired power station (Driscoll, Mahoney and Young,1973).

Cigarette and tobacco smoke is a curse on the environment that non-smokers have had to suffer. However, it is gratifying that with the passage of time there is an increasing concern for the right of everyone to breathe clean air. Sixty five per cent of British Rail coaches are now to be reserved for non-smokers. An occasion like this Symposium should not be allowed to pass without some reference to cigarette and tobacco smoke.

Oxides of nitrogen in cigarette smoke have been determined following scrubbing with 0.1M sodium hydroxide:

$$NO + NO_2 + 2OH^- \rightarrow H_2O + NO_2^- + NO_3^- \quad (g)$$

(NO_2^- oxidised to NO_3^- by MnO_4^-)

The emfs of the hydrolysate were determined before and after oxidation of nitrite to nitrate with an Orion 92-07 nitrate ion-selective electrode in the cell (Morie, Ledford and Glover,1972).

Cyanide (Vickroy and Gaunt,1970), hydrogen sulphide (Morie,1971), and ammonia (Sloan and Morie,1974) have been determined in cigarette smoke with the appropriate electrodes. Because of the low pH (5.4 to 6.4)ammonia exists as NH_4^+ ions, a fact supported by the non-removal of the protonated ammonia species by filters comprising of activated carbon. By collecting the ammonium-nitrogen in sodium hydroxide, it is possible to use the ammonia gas electrode for the determination (Table 3) and the levels obtained compare with those determined by gas chromatography (Sloan and Morie,1974). Methylamine interference is discounted since none was obvious in control runs with the compound added in amounts equal to the ammonium content: unspecified components in tobacco smoke and which interfered with the alternative Nesslerisation technique.

TABLE 3 Ammonia content of Tobacco and Smoke from Five Brands of Cigarettes (Sloan and Morie,1974)

Determination by ammonia gas electrode after extraction/absorption with sodium hydroxide.

Analytical sample	Ammonium nitrogen				
	Dark	Burley	Flue-cured	Non-filter Blend	Filter Blend
Cigarette tobacco/%	0.48	0.31	0.11	0.14(0.13)[a]	0.11
Smoke/μg cig.$^{-1}$	153	102	51	67 (67)[a]	36[b],38[c], 27[d]

a Acetate filter containing a polyol plus acidic additive
b Cigarettes containing 100 mg activated carbon in filters
c and d Cellulose acetate

Fluoride

The fluoride ion-selective electrode has been used even more than the nitrate electrode, but following the above coverage to various measuring situations it will not be necessary to enter into the same amount of detail here. Hence only a few examples will be considered.

Fluoride contamination from industrial complexes. Particulate and gaseous fluoride is known to be detrimental to plants and animals and frequently escapes into the environment from aluminium

smelters and phosphate fertiliser units.

Established methods for fluoride determination in vegetation are rather time-consuming depending on the preliminary ashing, alkali fusion, leaching or distillation stages adopted. Most laboratories previously depended on the classical Willard-Winter distillation-titration technique or the Belcher-Leonard-West alizarin colorimetric assay, but by now the fluoride ion-selective electrode has established itself for the final assay stage. The electrode is highly selective, and fluoride can be detected over a much wider range than for any spectrophotometric procedure, that is, about 0.02-1900 ppm. In addition, sampling techniques have been progressively improved so that a complete analysis is feasible within 30 minutes using the Schöniger oxygen flask method (Levaggi, Oyung and Feldstein,1971) compared with at least 4 hours for a sodium hydroxide fusion (Baker,1972) and around 2 hours for a more involved sodium carbonate-zinc oxide fusion (Luow and Richards,1972). However, vegetation can be successfully leached with perchloric acid (B.Vickery and M.L.Vickery,1976).

The average ground-level atmospheric fluoride levels around some aluminium smelters can reach 13 parts per 10^9 and Louw and Richards (1972) have analysed the fluoride contents of sugar cane growing in fields adjacent to such a complex using an Orion 94-09 fluoride ion-selective electrode. More fluoride was found in the leaves (0.82-3.46 ppm) and cane-trash (13.2 ppm) than in the stalks (0.16-1.96 ppm). After deliberate continuous fumigation of sugar cane with hydrogen fluoride (3.6 parts per 10^9 for 1 week followed by 17 parts per 10^9 for a further week) the levels for the terminal halves of leaves increased dramatically to 97.1 and 181 ppm, respectively, compared with the very low accumulations of 0.31, 0.32 and 0.34 ppm in the lower, middle and top sections in the stalk (Luow and Richards,1972).

Mention has been made of the use of TISAB in conjunction with the fluoride ion-selective electrode. This contains 0.001M sodium citrate in order to overcome interference by complexing metal ions, but the level of aluminium and iron can be very high in some plants and the tea plant is known to accumulate up to 17 000 ppm. By using a 0.25M sodium citrate buffer solution for extracting the ash of commercial tea samples containing 2050 and 2800 ppm respectively of aluminium and iron, recoveries of greater than 90 per cent fluoride may be obtained when determined with a Corning fluoride ion-selective electrode (B.Vickery and M.L.Vickery,1976).

Water. Environmental fluoride is a double-edged sword and while high levels ($>$1.2 to 1.5 ppm in potable water) causes damage, too little promotes dental caries. It was when the concern over monitoring fluoride in water supplies was at its highest that the fluoride ion-selective electrode was discovered (Frant and Ross, 1966). Although many papers have been published on the monitoring of fluoride in water (see Moody and Thomas,1973,1977a,1978), frequently concerning interference problems, the technique with the fluoride ion-selective electrode is generally elegantly simple and rapid and involves 1:1 dilution of the sample and standards with TISAB before measurement (Frant and Ross,1968).

Anaesthetic metabolism. Concern with the environment arises because pollution, whether deliberate as in emissions into the atmosphere, discharge into waterways and dumping of solid waste or unintentional as in the use of insecticides and fertilisers, leads to various acute or chronic toxicities that can also have legacies of changes in the laws of nature. Of course, such concern doesn't stop at pollution, for we are ever conscious of the short term and long term side effects of drugs, food additives, and even of anaesthetics that have been of such great benefit to mankind. Concern over anaesthetics has been brought into greater prominence since 1960 by the introduction of a series of fluorinated methyl ethyl ethers and similar materials into clinical practice. Cohen(1971) has reviewed the metabolism of many of these materials along with other halogenated and non-halogenated hydrocarbons.

Renal failure has been reported following human administration of methoxyflurane as an anaesthetic (Taves and co-workers,1970) and the effect is dose-related (Cousins,Nishimura and Mazze, 1972), the severity of disfunction being proportional to serum inorganic fluoride (Mazze, Trudell and Cousins,1971). Less than 50 per cent of the chemical is exhaled (Holaday, Rudofsky and Trehaft,1970), the remainder appearing as serum and urinary metabolites, among which is ionic fluoride and probably the nephrotoxin (Mazze, Cousins and Kosek,1972). Various metabolites have been determined in urine, among which 2,2-difluoromethoxy-acetic acid (after Schöniger oxygen flask combustion) and free inorganic fluoride are conveniently evaluated with the fluoride electrode.

This further emphasises the wide range of samples in which ion-selective electrodes have been used and urine is not by any means the simplest of body fluids for the conditions of pH, sodium levels and ionic strength vary considerably, but 1:1 dilution with chloride-free TISAB can generally cope with this.

The use of fluorine-based anaesthetisia is not the only reason for monitoring urine for fluoride and atmospheric fluoride levels exceeding 4-6 ppm demand surveillance of personnel and working conditions (Neèfus, Cholak and Saltzman,1970).

CONCLUSION

Just two ion-selective electrodes have served to illustrate the applications of ion-selective electrodes in environmental and toxicological analysis with just a mention of certain others, and gas sensors. They have been adequate to demonstrate how the main problems and sample variations may be overcome, but, of course, in such a brief discussion it has not been possible to anywhere nearly cover the subject completely. For a more extensive access to literature references the reader is invited to refer to fuller reviews (Moody and Thomas, 1971a-1973,1977a and b,1978).

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Continuous Trace Level Analysis Using Ion Selective Electrodes

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ABSTRACT

Continuous on-line analysis using ion selective electrodes require, in most cases, a continuous addition of multi-purpose reagent to establish the suitable measuring conditions. The traditionally used peristaltic pumps need a sample filtration and intensive maintenance to ensure the correct metering of sample and reagent. In addition ultra-pure reagents have to be used to prevent sample contamination. This paper describes the use of passive reagent addition systems, based on diffusing the reagent through a reagent permeable membrane and on dissolving a sparingly soluble reagent into the sample stream. This way the precise mechanical metering of sample and reagent stream and the need for ultra-pure reagents is not required. The passive reagent addition systems are mechanically simple, and thus highly reliable.
A sodium on-line monitor described in this paper, using passive reagent addition systems, exhibited a lower limit of detection of 0.1 ppb Na.
A similar on-line monitor for total residual chlorine exhibited a lower limit of detection of 1 ppb Cl_2. This system could also be used for on-line ozon determinations in tapwater.

KEYWORDS

Ion selective electrodes, on-line monitors, reagent addition systems, sodium determinations, total residual chlorine, potentiometry.

INTRODUCTION

Ion selective electrodes (ISE) have some favourable properties for the use in continuous on-line trace level analysis, like:

- The response is not affected by color, turbidity, and sample weight or volume.
- The logarithmic response to activity changes offers a constant relative accuracy over the entire measuring range until close to

the limit of detection
- The low limit of detection, typical 10exp(-6) to 10exp(-8) molar for most solid state ISE.
- Mechanically robust and easy to handle.
- Simple electronics is required.

On the other hand the following problems have to be overcome in order to obtain reliable results:

- ISE respond to ion activities while the desired information is mostly required in concentration units. Since the major factor influencing the difference between activity and concentration is the ionic strength. It is necessary to work at a constant ionic strength in order to measure concentration.
- ISE determinations are, like other analytical methods, subject to interferences depending on other constituents of the sample. These interfering species have to be removed prior to the measurement.
- The species of interest may not be in a form sensible by ISE eg. the species can be present in complex with or bound to other chemical components, and requires to be decomplexed prior to the measurement.

Under laboratory conditions the above mentioned problems are solved simply by the addition of a multi-purpose reagent to both sample and standards. This simple action under on-line conditions is traditionally done by precisly metering sample and reagent flow. In addition, small-bore plastic tubing is used to reduce the reagent consumption to an acceptable level. Consequently sample filtration is required to reduce the chance of blockage. These precise mechanical requirements are difficult to maintain over a long period of time. The resulting system reliability and the required maintenance time are the main reasons for the limited success of on-line analysers using ion selective electrodes.

In a number of cases the solution to these problems can be found in the use of passive reagent addition system, which is independent of the need for volumetric flow of both sample and reagent, and thus increasing the system reliability and decreasing the maintenance time.

PASSIVE REAGENT ADDITION SYSTEMS

Passive reagent addition systems based on the diffusion of a reagent into the sample stream has the great advantage of simplicity and negligible sample dilution. The design of these systems can be a short length of tubing immersed in a reagent reservoir. The sample flows through the tube and the reagent diffuses into the sample through the walls of the tube from the reservoir. (see fig. 1) The concentration of the reagent in the outward sample stream will be proportional to the permeability of the tubing to the reagent species, the concentration of the reagent species in the reservoir, the internal area of the tubing, and inverse proportional to the sample flow rate and tubing wall thickness. By varying tubing dimensions, it is possible in many cases to provide an optimum reagent concentration in the sample for a given flow rate.

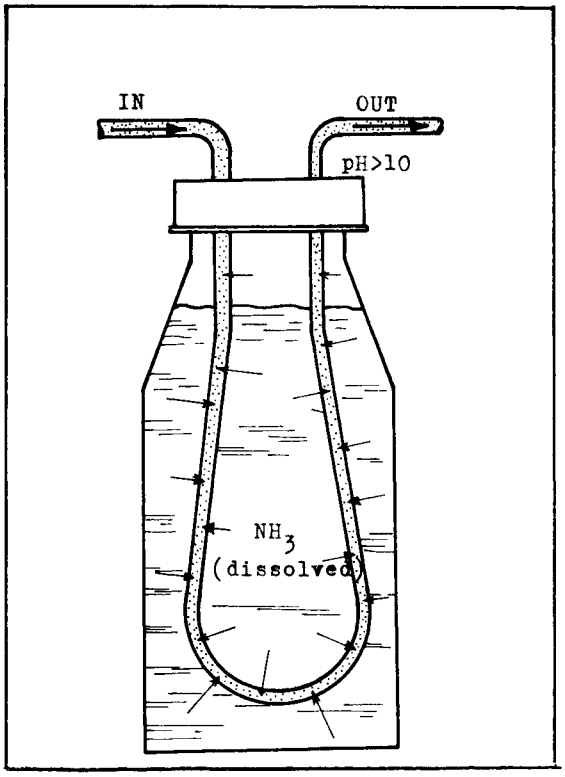

Fig. 1 Passive reagent addition system based on diffusion.

The chief limitation to this technique is the small number of available materials, which have sufficient permeability for the reagent species, and at the same time show sufficiently low permeability for the species being determined in the sample. The most useful materials at the moment are silicone rubber and porous Teflon. Silicone rubber is quite permeable to a wide variety of neutral, low molecular weight reagent species and impermeable to ionised species in the sample stream. PH control of a sample, for example, is easily accomplished by making use of the high permeability of silicone rubber to acetic acid and ammonia. Neutral oxidising agents, like bromine and iodine, can also be introduced. The diffusing rate of ammonia through the wall of a silicone rubber tube (outer diameter 5.5 mm, inner diameter 3 mm) immersed in a 25% (weight) ammoniumhydroxide is

7 x 10exp(-5) gmol NH_3 per minute per meter tube.

Porous Teflon tubing, which is not wettable by aqueous and many organic solutions, makes it possible to isolate the sample stream from the reservoir with a thin, contiguous air space contained within the walls of the tubing. Any reagent species with a significant vapor pressure can be introduced by diffusion across the air layer. The air layer is, of course, completely impermeable to ionic species in the sample. This is of great importance with respect to trace level analysis, where the purity of the reagent becomes a major problem.

Dissolving the required reagent in the sample stream is another method to achieve a flow independent, passive reagent addition system. The reagent should be a sparingly soluble salt, which can be compressed and formed into mechanically stable rods or pellets. The pellet or rod inserted in the sample stream will dissolve, resulting in an almost saturated solution. This method can easily be applied to those electrode systems, where a small variation of the reagent concentration does not effect the measuring result, like in the chlorine monitor described in this paper.

LOW LEVEL SODIUM MONITOR USING PASSIVE REAGENT ADDITION SYSTEM

A glass sodium electrode, under the right sample conditions, exhibits a linear response to as low as 10exp(-8) molar sodium according the adapted Nernst equation;

$$E = E_o(T) + \frac{RT}{nF} \ln A_{Na}$$

where; E is the potential of the electrode chain in mV,

$E_o(T)$ is the potential of the electrode chain at temperature T when A_{Na} equals unity,

R, T and F are resp. gasconstant, temperature (^oK) and Faraday's number,

n is an experimentally determined factor using known sodium solutions.

The only significant interference experienced by the sodium electrode is hydrogen ion. It is therefore necessary to raise the pH to a level that will minimize, if not entirely eliminate the hydrogen ion error. This is accomplished by using the permeability of silicone rubber tube to ammonia vapor. The sample stream passes through a silicone rubber tube of suitable length immersed in a concentrated ammonia solution. The pH of the entering sample can be pH = 2,5 and the pH of the existing sample will be 10,2 or higher. The length of the silicone rubber tube is choosen to meet the ammonia requirement under the highest flow conditions. The purity of the ammonia solution with respect to sodium is unimportant since no ions can pass across the silicone rubber. The life of 1 liter 25 % ammoniumhydroxide with a sample flow rate of 40 ml/min is approximately 12 months. Clearly, this approach provides a high degree of system reliability. (see Fig. 2 and 3)

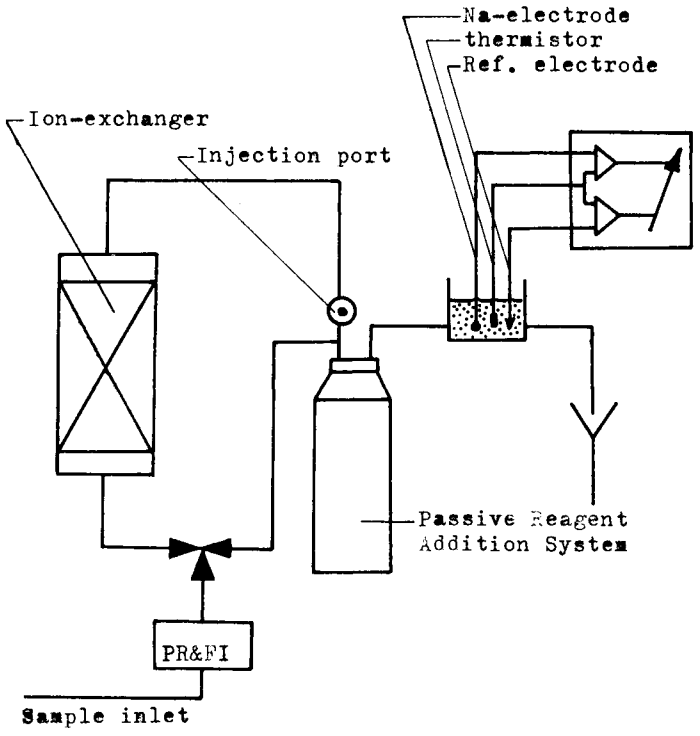

Fig. 2 Sodium Analyser Diagram

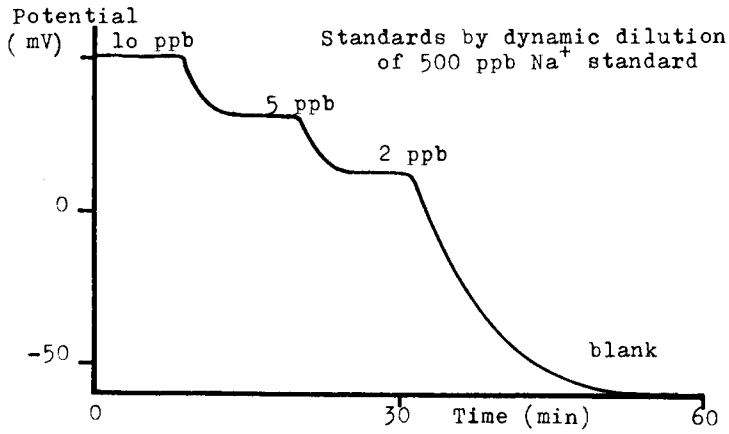

Fig. 3 Response of sodium monitor (Orion Research type a/SleD) to sodium concentration changes

A "TOTAL RESIDUAL CHLORINE" MONITOR USING PASSIVE REAGENT ADDITION

The "total residual chlorine" electrode (Orion Research Inc., Model 97-90) is a combination platinum-iodide electrode which senses the iodine released when chlorine reacts with iodide according:

$$Cl_2 + 2I^- \rightleftharpoons I_2 + 2Cl^- \qquad (2)$$

Followed by:

$$I_2 + I^- \rightleftharpoons I^-_3 \qquad (3)$$

and

$$I_2(\text{solution}) \rightleftharpoons I_2(\text{gas}) \qquad (4)$$

The reactions (3) and (4) will have a neglectable effect on the final iodine concentration by limiting the excess iodide level to less than 10exp(-3) molar and by measuring shortly after the iodide addition. These conditions are met with the monitor described in fig. 4. The iodine produced in a chlorine containing sample, after the addition of an excess iodide, will be equal to the chlorine present in the original sample.

The electrode response can be described as follows (1):

$$E = E_0\,(T) + \frac{RT}{2F} \ln\ I_2 \qquad (5)$$

Note: The electrode response is independent of the amount of iodide remaining after the reaction.

The needed acidification followed by the addition of iodide to the sample stream in order to use the electrode system for "total residual chlorine" measurements is done in two steps using a passive reagent addition system for each of them.

- The acidification of the sample stream is achieved by diffusing acetic acid through the wall of a silicone tube.
- The addition of iodide to the sample stream is done by dissolving the sparingly soluble leal-iodide. The acidified sample is passed along a mechanical stable rod of lead-iodide, and in passing a sufficient amount of iodide will dissolve to react stoichiometrically with 10 ppm of chlorine.

The analytical performance of this measuring system allows a lower detection limit of 1 ppb and an upper limit of 10 ppm.

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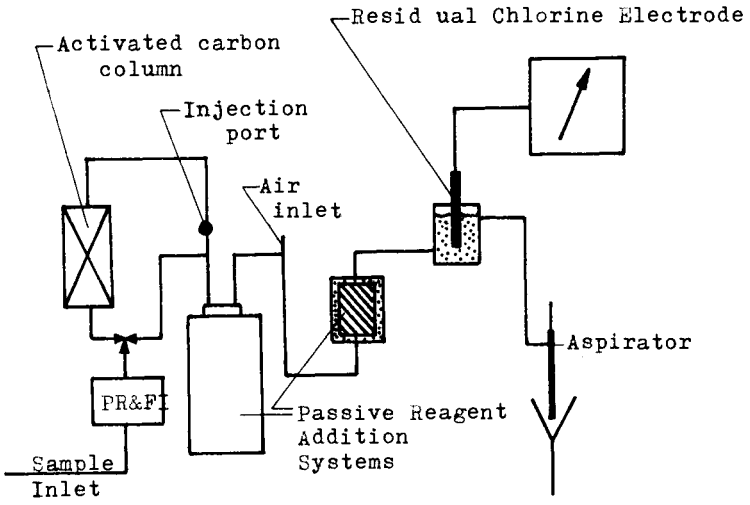

Fig. 4 Residual Chlorine analyser diagram

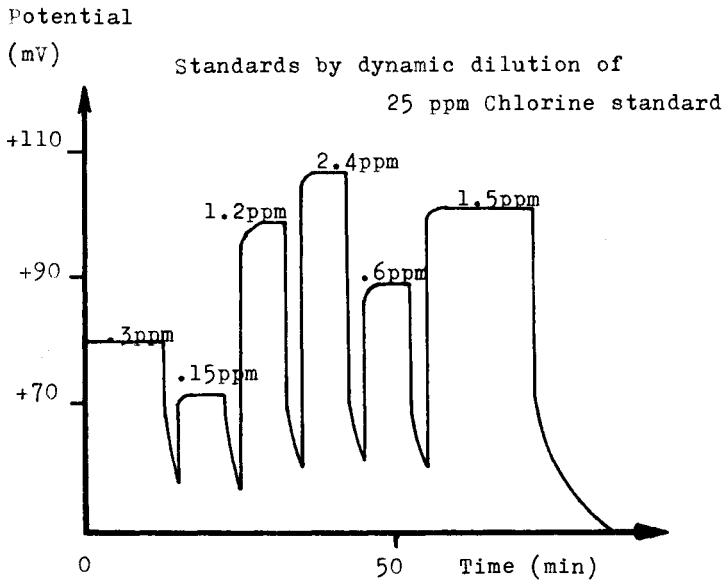

Fig. 5 Response of Chlorine Analyser (Orion Research type 1570) to Chlorine concentration changes

Fluoride Determination by Continuous Flow Analysis

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ABSTRACT

Three methods for the continuous flow determination of fluoride ions are described.
Firstly a colorimetric method using the alizarin-lanthane complex.
Secondly a direct potentiometric method making use of a fluoride ion-selective electrode and,
Thirdly the Gran addition method by ion-selective electrode.

The advantages and drawbacks are stressed. The unquestionnable technique is the Gran method. It allows the determination of the total fluoride content and also of the fluoride complexed by the medium.

KEYWORDS

Fluoride, Continuous flow analysis, Ion selective electrode, Gran method, Air pollution.

INTRODUCTION

Fair amounts of fluorides are discharged in the air by aluminium factories. (Dossier fluor 1976, Garrec,1977). The control of immissions by monitoring stations is the most reliable but the most expensive (Powell,1973). Solid absorbent captors can be used to compare immission levels (Wilson, 1967). According to Israel (1974, 1977) good correlations have been found between fluoride levels accumulated in plants and those collected on impregnated paper samplers.

We describe, in this article, three continuous flow techniques of analysis of fluorides, sampled according to the above procedure. The volume of air in contact with the filter papers is not known, instead one talks in terms of the determination of a fluoride flux, consisting mainly of HF or fluorine molecules. The captors are made of a vertical support on which are fixed ten filter papers impregnated with a solution of sodium silicate. They are protected against bad weather conditions. Every month the filter papers are collected and the

adsorbed fluoride determined in the laboratory.

Quantitative Determination Methods

Colorimetry. Fluoride ions can be determined colorimetrically by alizarin-methylamine-NN-diacetic acid (Analytical methods committee 1971). Continuous flow methods of analysis have been described (Technicon 1972, 1974). Straight line graphs for concentrations up to 4 μgF/ml were obtained. Past experience, in the framework of the control of immissions brought about by three aluminium factories, shows that concentration values of between 0.5 and 70 μgF/ml are common. We have constructed a manifold which allows the successive determinations of solutions of concentrations ranging from 0.5 to 10 μgF/ml and 5 to 70 μgF/ml. No clean up is necessary as the analytical medium is simple.

Determination by ion-selective electrode. Frant (1966) constructed an ion-selective electrode for the determination of fluoride ions. Automated methods for the determination of fluoride in water have been published (Erdman, 1975; Bystrova, 1976). To verify the results obtained by colorimetry we have devised a potentiometric method of analysis making use of an ion-selective electrode. It allows us to measure concentrations in the range of 0.5 to 70 μgF/ml. It is simpler and less expensive than the colorimetric method which furthermore gives no information on the complexing power of the medium towards fluoride ions.

A continuous flow potentiometric method making use of the Gran method is described. It allows the determination of concentrations from 1 up to 100 μgF/ml. It is therefore possible to determine complexed fluoride, free fluoride and total fluoride concentrations.

EXPERIMENTS AND METHODS

Preparation of Samples

The 10 filter papers, after sampling, are transferred to a 400 ml large neck polyethylene bottle. 250 ml of water are added and the whole left to stand overnight. After agitation, an aliquot part is filtered into the measuring cuvettes.

Colorimetric Method

Alizarin-3-methylamine-NN-diacetic acid forms a red complex with lanthane(III) which combines with fluoride ions to form a blue 1:1:1 ternary complex at pH 4.6. The concentration of fluoride is determined by absorption at 630 nm.

Apparatus and reagents. The Carlo Erba continuous flow analyser comprises a sample distributor SD3 model 1512, a proportioning pump PP20 model 1512/20, a reactor model 1514, a colorimeter model DCM 1530 and a 601 W+W recorder. All reagents used are of the analytical reagent grade.

Manifold for colorimetric measurements. The manifold consists of 2 distinct parts. Fig. 1. The first allows the direct determination of solutions of low concentrations (0.5 to 10 μgF/ml). The pH of the sample is adjusted to 4.6 with a succinic acid-succinate buffer solution. The $2.2 \cdot 10^{-3}$M - $2.0 \cdot 10^{-3}$M alizarin-lanthane reagent is then added. The mixture then goes into the

colorimetric cell A for measurement.

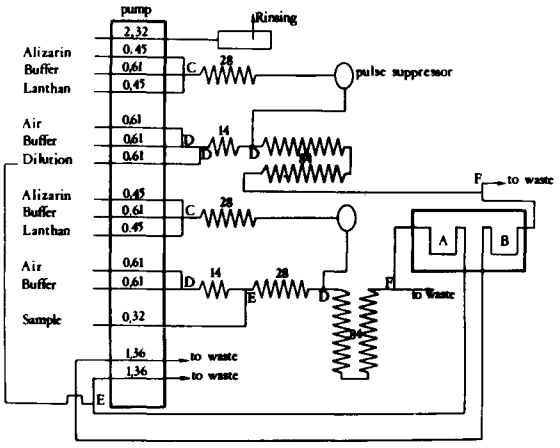

Fig. 1. Manifold for colorimetric measurements. Under "pump": flows ml/min. A and B photometric cells.

The second part dilutes the reaction mixture. A fraction of the solution coming out of cell A is diluted with the buffer solution. The diluted mixture then flows through a circuit similar to circuit one before going through the photometric cell B.

The sampling time is one minute and the complete cycle of analysis lasts three minutes.
After a day's operation it is necessary to dissolve away the alizarin-lanthane complex which may have deposited on the walls of the vessel with a solution of NaOH 0.5M.

Ion-Selective Electrode Method

Apparatus. The Carlo Erba sample distributor and proportioning pumps are used. The determination is performed by means of a Beckman ISE no 39600, a Metrohm EA 425 Ag/AgCl reference electrode saturated with KCl. The potential is measured by means of a Metrohm E500 digital voltmeter.

Manifold and procedure. Two operations are performed by the manifold Fig. 2.

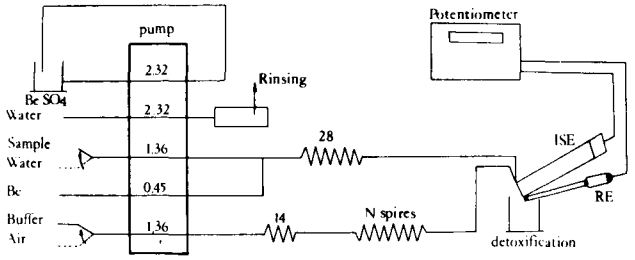

Fig. 2. Manifold for potentiometric measurements.

The sample is buffered acidwise and ionic strengthwise by means of a 1M acetic acid-acetate solution of pH 5.3. A retarding circuit with a 0.1M beryllium sulphate solution of pH 5.3 allows the rinsing of the electrode surface soon after the passage of the sample. In this way any fluoride which adhered to the membrane surface is desorbed. A quick rinse with buffer solution then follows.

For the measurement of potential with small flows, two cellulose acetate trips are glued on the electrode surface leaving a path about 1 millimetre wide in the centre. The whole surface is then treated with a surfactant.

The ion-selective electrode and reference electrode are placed one above the other. The solutions flow freely over the electrodes from the top.

The time of sampling of the fluoride and beryllium solutions lasts 2 minutes, and the complete cycle of analysis lasts 4½ minutes.

Method of Additions, Measurement by Ion-Selective Electrode

Apparatus. The same set up as for simple potentiometric analysis is used. In addition three minimicro 2/6 Ismatec peristaltic pumps are coupled to 3 Selectron GPR 32 relays which under the given impulse of the sampler, performs at preselected times three additions of reference solutions.

Manifold and procedure. The manifold Fig. 3, draws a sample and adjusts its pH and ionic strength by means of a 1M acetic acid-acetate buffer solution of pH 5.3. Three peristaltic pumps perform the additions of the buffered (pH 5.3) standard solutions. It is necessary before performing a series of measurements to measure the flow of each solution. The slope of the ion-selective electrode must be known.

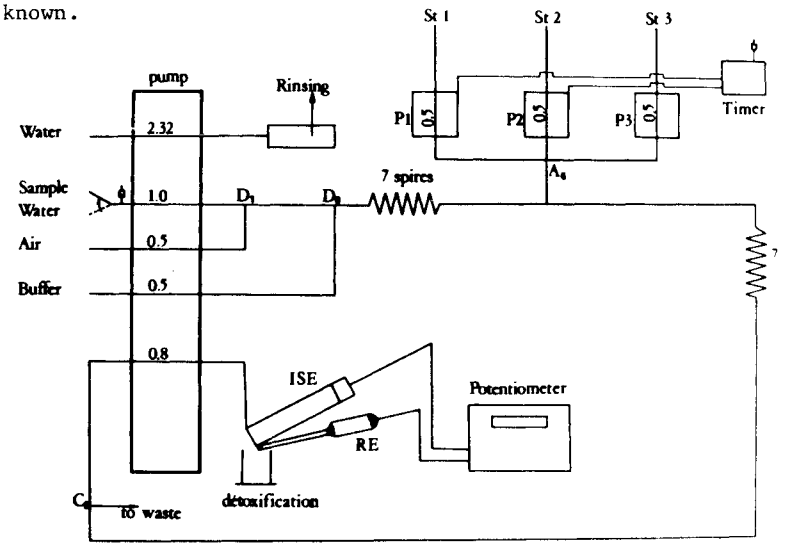

Fig. 3. Manifold for Gran addition method.
P_1, P_2, P_3, additional pumps.

RESULTS AND DISCUSSION

Colorimetric Method

Landry (1978) discussed the effects of reagent concentrations and auto-absorption phenomena: It follows that a dilution of the reacting medium is possible. Two straight line graphs are therefore obtained, one for the range 0.5 to 15 μgF/ml. and the other for the range 5,0 to 70 μgF/ml. Under the analytical conditions, the presence of sodium silicate (1 g/l) brings about a 12.5% slope reduction in the calibration graph.

Potentiometric Determination

Theory. The potential of the fluoride ISE is given by the equation:

$$E = E^{o} + E_j - E_r + s.\log \alpha . \gamma_F .c'(F) \quad (1)$$

where E^{o} and E_r are the standard electrode potential and reference electrode potential respectively. γ_F is the activity coefficient of the fluoride ions, c(F) and c'(F) are the free and total concentration of fluoride respectively. s is the slope of the ion-selective electrode. α the complexing ratio is given by: $\alpha = c(F)/c'(F)$ (2)

The junction potential has been reduced to a minimum by using a 1M solution of KCl in the reference bridge. The ionic strength value of 1 of the buffer solution (pH 5.3) keeps the value of the activity coefficient constant. In the continuous flow addition method a known flux ϕ of concentration C is added to an initial flux ϕ_o of the unknown solution c'_o. This is buffered by a solution of flux ϕ_t. Under equilibrium conditions one obtains:

$$(\phi_o + \phi_t + \phi)\exp(E/s) = \alpha . \gamma_F .\phi_o . c'_o(F).\exp(E^{o}/s) + \alpha . \gamma_F .\phi.C'(F).\exp(E^{o}/s) \quad (3)$$

This is of the form

$y = p.x + q$

where $p = \alpha . \gamma_F .\phi.\exp(E^{o}/s)$ $\quad q = \alpha . \gamma_F .\phi_o .c'_o(F).\exp(E^{o}/s)$ (4)

By means of these equations α can be calculated. Comparing p_1 or p_2 for two different states "1" and "2", one being a reference state, one gets the following equation: $p_1 . \alpha_2 = p_2 . \alpha_1$ (5)

For the HF/F^- system equation (5) gives $p_2 - p_1 = K_s / \gamma_F .(p_1 .a_1(H) - p_2 .a_2(H))$ (6)

where a(H) is the activity of the hydrogen ion and K_s the thermodynamic stability constant of HF.

By putting $p_2 - p_1 = f\ (p_1 .a_1(H) - p_2 a_2(H))$ one obtains a straight line graph of slope K_s / γ_F.

Electrode response. The electrode response is such that when changing from a concentrated solution to a dilute solution the measured potential does not correspond to the equilibrium potential. Fig. 4 shows that a quantitative analysis is impossible, the error can be as high as 37%.

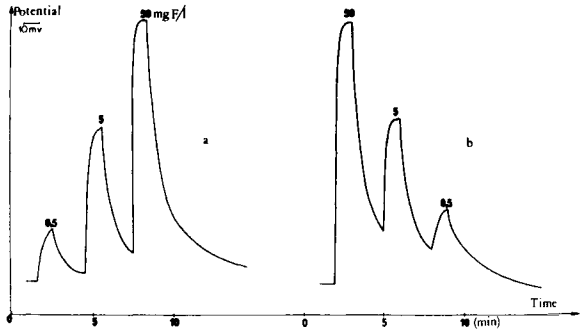

Fig. 4. Response of ISE in various conditions.

Buffle (1974) showed that the adsorption of fluoride ions at the solution-membrane interface reduces the sensitivity of the ion-selective electrode. We have shown that a rapid return to the baseline potential is possible by complexing the adsorbed fluoride. Ringbom (1967) showed that of the following cations, La(III), Cr(III), Th(IV), Al(III) and Be(II), the latter is the most efficient complexing agent. Experimentally, for 10^{-3}M solutions of the above mentioned cations and a 10^{-4}M solution of F^-, Be(II) proves to be a better complexing agent of F than Al(III). The stability constant of the complexes is in the order: Be(II) > Al(III) > Th(IV) > Cr(III) > La(III) > H_2O. Be(II) being weakly hydrolysed makes it easier to work at a pH value that is not very acid. Experience shows that during the rinsing step of the ion-selective electrode membrane the response time is considerably reduced as the concentration of Be(II) increases. The baseline potential is recovered in 3.2 minutes when a 0.1M Be(II) solution is used.
The results obtained using the manifold Fig. 2 are shown in Fig. 5.

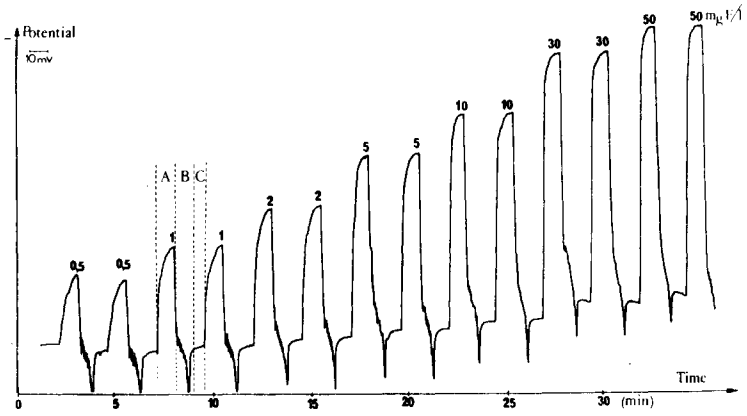

Fig. 5. Sequence of analysis.

In the first part of the graph (A), the electrode responds to the fluoride solution. In the second part (B), the rinsing solution and the 0.1M $BeSO_4$ solution reach the electrode surface simultaneously. In part three (C), only the rinsing

solution of pH 5.3 flows over the membrane. There is hardly any change in the baseline potential. Under these conditions the calibration curve is linear up to 1 μgF/ml with a slope of 63 mV. It is possible to extend the linearity of the calibration curve by performing measurements at arbitrary times.

Gran Method

Under the experimental conditions two concentration scales afford the determination of fluoride in solution.
1) from 0.5 to 10 μgF/ml with standard solutions of 5, 10 and 15 μgF/ml
2) from 5 to 100 μgF/ml with standard solutions of 50, 100 and 150 μgF/ml.

There is an excellent agreement between experimental and theoretical values. The correlation coefficient is as good as 0,9999 and the error at the origin is 0.2 μgF/ml. With a view to reducing the analysis time we have studied the response kinetics of the ion-selective electrode.

Parthasarathy (1978) showed that it could be represented by a hyperbolic function :

$$\frac{E.t - E_i.t_i}{E - E_i} = E_{eq} \frac{t - t_i}{E - E_i} - \frac{B}{A} \qquad (7)$$

Where E_{eq} corresponds to the equilibrium potential, E to the potential at time t, E_i the potential at the beginning of the time t_i. A and B are numerical constants. Using this equation by analogy with the continuous flow determination (3) we have obtained straight line graphs with slopes greater than the slopes corresponding to arbitrary time graphs Fig. 6. The concentration $c_o'(F)$ is not influenced by this phenomenon. On the other hand, for the calculation of α the value of the potential at a time t must be corrected.

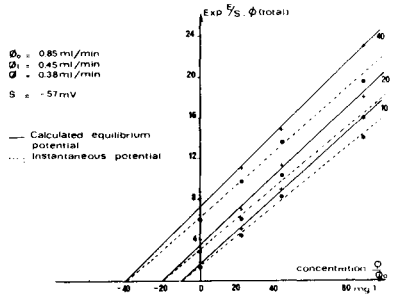

Fig. 6. Deviation of arbitrary time graphs from calculated equilibrium time graphs.

Determination of the stability constant K_s of HF. The experimental results substituted in equation (6) leads to the following expression :

$$p_2 - p_1 = 2.1 \cdot 10^3 (p_1.a_1(H) - p_2.a_2(H)) - 0.028$$

with a correlation coefficient of 0.93. The calculated apparent constant is equal to $2.1 \cdot 10^3$.

Comparison of the Colorimetric and Potentiometric Methods

Table I. shows the results obtained for simultaneous measurements of solutions prepared from sodium silicate impregnated filter papers which were exposed over a period of 30 days in the Rhône valley between Steg and Martigny. The measurements by simple potentiometry give systematically lower values than the colorimetric method. On the other hand, the values are systematically higher by the Gran method. This systematic error originates from the complexing of fluoride ions by silicates in the direct potentiometric method. The latter case is not exemplified by the colorimetric method. As for the Gran method it allows the determination of total fluoride concentrations irrespective of the complexing ability of the medium.

TABLE I Comparison of the Three Methods

	CONCENTRATION F (MG/L)		
	COLORIMETRIC	POTENTIOMETRIC	GRAN
NO 1	14,8 ± 0,4 *	15,0 ± 0,5	15,5 ± 0,5
2	9,4 ± 0,2	9,0 ± 0,5	9,1 ± 0,3
3	8,4 ± 0,2	8,3 ± 0,5	8,3 ± 0,3
4	5,5 ± 0,2	5,2 ± 0,5	5,6 ± 0,2
5	4,5 ± 0,2	4,2 ± 0,5	4,9 ± 0,2
6	2,0 ± 0,2	1,8 ± 0,5	2,3 ± 0,2
7	21,2 ± 0,4	21,0 ± 1,0	22,6 ± 1,0
8	37,2 ± 0,4	36,0 ± 2,0	37,2 ± 1,[illegible]
9	127,0 ± 0,8	125,0 ± 4,0	124,0 ± [illegible]
10	45,8 ± 0,4	43,0 ± 2,0	[illegible]3,3 ± 2,[illegible]

* Standard deviation

CONCLUSION

Continuous flow measurements have been practised since the month of june 1976. The fluoride concentrations of silicate impregnated filter papers have been monitored since 1965 for different localities of the Valais. No discrepancies have been found between the old and existing methods.

By extension, the Gran potentiometric method has been applied to various media not requiring mineralisation. Excellent results have been found in the case of soft drinks, wine, alcohols, milks, etc. Tooth paste with or without fluoride have also been treated by this method.

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The Use of Copper Ion-selective Electrode for Determination of Copper Chemical Forms in Natural Waters

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ABSTRACT

Copper may be present in natural waters both associated with suspended solids and in different soluble chemical states. An analytical scheme, based on the use of cupric ion-selective electrode, has been devised to quantitatively differentiate the soluble inorganic and organic cupric species. The inorganically bound copper is mostly present as hydroxo- and carbonate complexes and after measuring equilibrium free cupric ion, pH and total carbonate concentration may be easily calculated. Organically bound copper soluble fraction mostly originates from copper interaction with humic acids. Copper in this form may neither be calculated from measured equilibrium free cupric ion nor be set completely free and hence measured at low pHs: after calibration it was shown that it may be correctly evaluated by extrapolating to pH 3 the concentration measured as a pH function.

Keywords: copper in natural waters; copper speciation; copper ion-selective electrode, use of; apparent complexing capacity of waters; carbonate and hydroxo-species in copper speciation; distribution of copper among inorganic complexes and organic species in waters.

INTRODUCTION

The need to better understand the transport and biological interaction of heavy metals in natural waters has made the study of their chemical speciation a subject of primary interest in analytical chemistry research. The ion-selective electrode is presently one of the most precious analytical tools when dealing with speciation problems, due to its ability to differentiate between the free ion and the complex species: it was applied by many authors (Stiff, 1971; Smith, Manahan, 1973) to the determination of cupric ion at submicromolar concentration levels. Total copper in slightly polluted or unpolluted river waters is in the range of few ppb; only a very little fraction, sometimes

less than 1%, is in the form of free ion. We found that using the Orion 94-20 A ion-selective electrode, even such low concentration can be measured with a 20% reproducibility, provided a careful calibration was set up. We restricted our investigations on copper in natural fresh waters to the soluble matter that, after Stiff (1971), may be defined as that which passes a membrane filter with 0.45 μm pore size and includes copper both as free ion and as soluble inorganic and organic complexes. It is possible that colloidal matter which includes polypeptide material, humic acids and some clays and metallic hydroxides might exist in part in a finely divided state that would pass the filter and therefore would be defined as soluble. Our experiments gave a strong evidence that even the Pellicon PT filter of nominal molecular weight limit (NMWL) 10^3 does not retain all colloidal matter and the water passing this filter does not represent a true solution. The filtration through a membrane filter with 0.45 μm pore size is anyway accepted as the way to separate suspended particles from soluble matter.

EXPERIMENTAL

Copper Ion-Selective Electrode Calibration

The free cupric ion was measured with the ion-specific electrode Orion 94-20 A coupled to a single junction reference electrode, both connected to a high impedence Orion potentiometer. The electrode needed to be carefully calibrated before to be routinely used (Hansen, Lamm, Ruzicka, 1972; Smith, Manahan, 1973; Blaedel, Dinwiddie, 1974; Midgley, 1976). A set of operational conditions was fixed to insure a good reproducibility and to make sure that the electrode response was proportional to the actual Cu^{++} activity in the pH range 9 to 4. Ionic strenght was fixed to 0.05 with KNO_3, oxygen interference was avoided by adding formaldehyde up to 10^{-3}M, stirring speed and electrodes distance were kept constant. TPX plastic containers were used avoiding glass and polietylene from coming in contact with diluted copper solutions. Suprapure $Cu(ClO_4)_2 \cdot 6H_2O$ was dissolved in tridistilled water to prepare a standard 10^{-1}M copper solution. More diluted solutions, up to 10^{-5}M, were prepared from the former. According to an extensive investigation on this subject (Hansen, Lamm, Ruzicka, 1972) solutions with (Cu^{++}) lower than 10^{-5}M were prepared as copper ion buffers with nitrilotriacetic acid (NTA) in sodium acetate solution (pH = 4.75) for (Cu^{++}) as low as 10^{-9}M, and in borax solution (pH = 8.80) for (Cu^{++}) as low as 10^{-13}M. When necessary KNO_3 was added to adjust the ionic strength to 0.05. Calibration graphs showed that the electrode response is linear down to 10^{-12}M, thus covering the range we were interested to. Concentration instead of activity was in our experiments plotted versus the mV response as concentration is directly known or calculated in the samples used to calibrate. Accurate measurements of pH were simultaneously carried out with conventional pHmeter.

Analytical Procedure for Inorganic Copper

In our experiments each water sample, after measuring the pH, was passed through a Millipore HA filter (0.45 μm pore size) under a 1.5 atm N_2 pressure. One aliquot of the filtered solution was potentiometrically titrated with HCl to measure the alcalinity and hence the total carbonate carbon, C_T, was deduced as suggested by Stumm (1970). At natural pH levels C_T is only slightly higher, about 5%, than alkalinity. Then pH and cupric ion concentration were measured. As already shown in a previous paper (Stella, Ganzerli-Valentini, 1978) the most common inorganic copper species in a fresh water are complexes or ion pairs containing OH^- or CO_3^{--} ligands. To calculate the complex distribution we used the method already applied by other authors (Stumm, Morgan, 1970; Zirino, Yamamoto, 1972; Baudouin, Scoppa, 1974): it is required to known the total free ligand concentrations which may be deduced from pH and C_T and the conditional stability constants, which are easily calculated at the actual ionic strenght (I = 0.05) from the absolute values reported in the literature (Sillen, Martell, 1964, 1971). Other inorganic ligands such as chloride, cyanide, sulphide, phosphate and poliphosphate ions could interfere. Their influence was tested and found negligible at least at the levels found in our samples. Similarly the concentration of soluble organic ligands such as citrate and aminoacids has to be considered negligible. Therefore we restricted our considerations to the following equilibria:

	log K (I = 0.05)
$Cu^{++} + H_2O \rightleftharpoons CuOH^+ + H^+$	- 7.52
$2Cu^{++} + 2H_2O \rightleftharpoons Cu_2(OH)_2^{++} + 2H^+$	-10.54
$Cu^{++} + CO_3^{--} \rightleftharpoons CuCO_3(aq)$	6.04
$Cu^{++} + 2CO_3^{--} \rightleftharpoons Cu(CO_3)_2^{--}$	9.28

A graphic rapresentation of all species in equilibrium at various pHs, in the range 9 to 6, is reported in Fig. 1 as a specific example. Inorganic copper speciation may thus easily be obtained after measuring only three parameters: pH, C_T and (Cu^{++}).

Analytical Procedure for Organic Copper

Copper reacts with the miscellaneous organic ligands in water because of its strong and non specific association with nearly all ligands, but as humic acids represent the largest fraction of soluble or colloidal organic matter in natural waters, it is likely to consider that most of the trace copper is "taken up" by them. They originate from decaying vegetable matter and carry carbonyl and hydroxyl functional groups. The soluble fractions of the humic acids are more properly called fulvic acids: for the sake of semplicity the term "humic acids" is hereafter used to indicate both species. It is not yet

certain whether such substances can really keep metals in solution or form highly dispersed colloids: it is anyway impossible to theoretically calculate, from known equilibrium free copper, the bound fraction as the reaction mechanisms are very complex and stability constants are unknown.

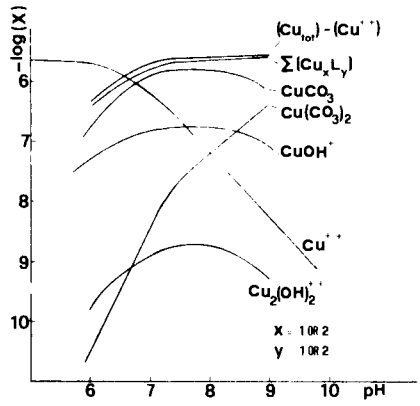

Fig. 1. Copper carbonate and hydroxo- complex distribution as a pH function.
$(Cu_{tot}) = 2.50 \times 10^{-6}$M, $C_T = 2.62 \times 10^{-3}$M

Our first attempt to overcome these difficulties consisted in studying the system as a pH function, by adding to the filtered samples Suprapure $HClO_4$ and measuring the corresponding free cupric ion concentration. Even humic acid complexation reactions are in fact pH dependent: dissociation of the complexes is favored at low pHs due to the competition of hydrogen ions for the ligands. Results reported in side A of Fig. 2 refer to artificial samples prepared by adding measured copper aliquot to solutions of pure humic acids (h.a.), supplied by ICN-K & K Laboratories, USA.

Results show that the electrode response starting from natural pH first increase sharply, then tend to flatten and below pH 4.2 tend to decrease. From this point in fact the humic acids begin to polimerize and give larger aggregates that may also precipitate causing copper absorption. This new phenomenon subtracts cupric free ions, that for this reason by no way may be completely set free. Total amount may only be obtained by extrapolating to pH 3. This was proved on several artificial samples and the extrapolation procedure was thereafter applied to actual ones. By subtracting from the total copper concentration the inorganic fraction previously estimated the organic fraction, including also colloids, may be deduced.

Copper offers the possibility to measure the apparent complexing capacity (ACC) of natural waters because the reaction of ionic copper added to water includes direct coordination as well as substitution of other metals from

their complexes (Chau, Gachter, Lum-Shue-Chan, 1974).

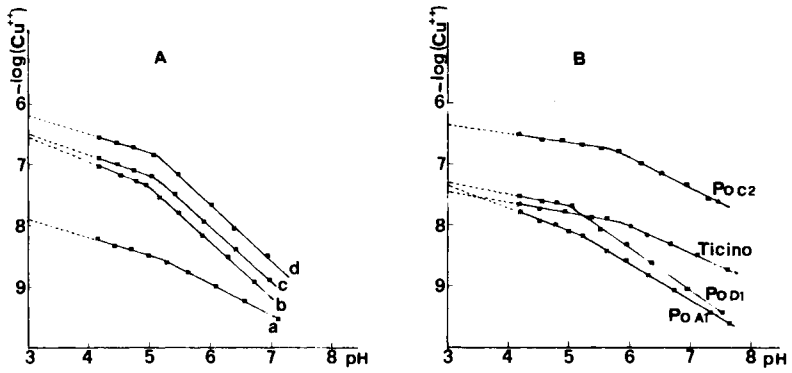

Fig. 2. The influence of humic acids on free cupric ion equilibrium concentration as a pH function; A - artificial samples: added copper 1.2×10^{-8}M in a, 2.8×10^{-7}M in b and c, 6×10^{-7}M in d; h.a. 5.25 mg/l in a, c and d, 10.5 mg/l in b. B - River water samples.

If the addition of cupric ions is made at natural pH inorganic ligands also subtract copper which finally may precipitate as malachite $Cu_2(OH)_2CO_3$, on the other hand added copper can not be expected to distribute proportionally to the natural pattern. It is in fact impossible to reproduce the conditions which lead to the natural balance. For these reasons we measured the apparent complexing capacity at pH 5.5 at which the contribute to complexation of hydroxyl and carbonate ligands is negligible. In a paper previously mentioned (Chau, Gachter, Lum-Shue-Chan, 1974) it is also shown that the apparent complexing capacity is independent of the original pH in the range 4-8. Experiments carried with a Po river sample are reported in side B of Fig. 3, where the observed equilibrium free cupric ion concentration is reported as a function of copper spike, the total added cupric ion amount. The slope of the curve changes and becomes stable at 1 at the ligands saturation point.

RESULTS AND DISCUSSION

The described procedure was applied to water samples collected from Ticino and Po rivers in the course of an interdisciplinar investigation on their ecosystems. The samples were immediately run when possible and stored at 4°C in the case the analysis had to be delayed. The trend of the adicification curves of the river waters, reported in Fig. 2, side B, is very similar to that found for artificial samples of pure humic acids (Fig. 2, side A). This indicates that copper in natural systems is taken up by humic acids or by other colloidal matters which similarly behaves. Results reported in Table 1 refer to extreme situations we found in a large serie of samples. In the last

column, indicated as ACC, the apparent complexing capacity is reported.

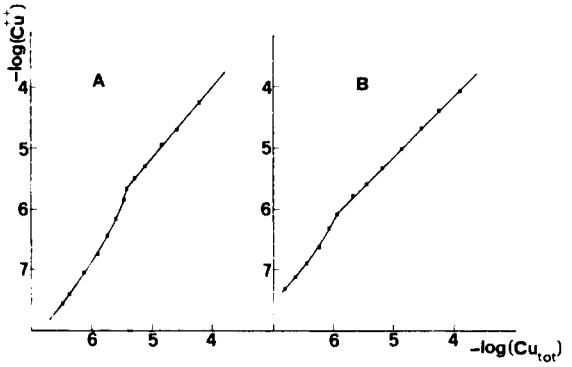

Fig. 3. Determination of the apparent complexing capacity of organic ligands. A - artificial samples of 5.25 mg/l of h.a.; B - A Po river sample

In the upper part of the Table the directly measured values are presented. The calculated concentrations of the different species considered are reported in the lower part and show that the predominant species is the ion pair $CuCO_3^0$. The $Cu_2(OH)_2^{++}$ and $Cu(CO_3)_2^{--}$ species account for a negligible fraction of total copper. The total organic copper, which represents a very large fraction, is indicated as CuL_{org}: it is simply obtained as a differente between the total copper evaluated at pH 3 and the sum of all inorganic copper specie concentrations. The procedure illustrated in this work presents the advantage of giving a rather satisfactory representation of copper speciation with a restricted number of measurements. The precision was tested by running several samples for total copper by atomic absorption: results agreed within 20% with the copper ion selective electrode data.

This method fails to give reliable results if consistent amounts of cyanides, sulphides and soluble silicates are present. In these cases in fact copper cannot completely be released by moving the pH toward lower values and consequently it is possible only to evaluate the labile inorganic species (carbonate and hydroxo complexes) as their concentrations are simply related to the pH and the cupric free ion concentration.

Researches are also in progress to provide informations, using ultrafiltration techniques, on copper distribution as a function of particle size of dispersed colloids in the organic copper fraction. A careful standardization of the ultrafiltration technique is necessary to overcome problems such as adsorption losses and trapping of particles in the pores of filters.

TABLE 1 Copper Species Distribution in Natural Waters

Sample	pH	C_T^*	(Cu^{++})	(Cu_{tot})	ACC**
Po A1	7.63	1.71×10^{-3}	2.3×10^{-10} 0.5%	4.6×10^{-8}	0.5
Po C2	7.48	1.85×10^{-3}	2.3×10^{-8} 5.1%	4.5×10^{-7}	0.4
Po D1	7.52	2.59×10^{-3}	3.8×10^{-10} 0.8%	5.1×10^{-8}	0.4
Ticino	7.62	2.08×10^{-3}	1.8×10^{-9}	3.6×10^{-8}	0.3

Sample	$(CuOH^+)$	$(Cu_2(OH)_2^{++})$	$(CuCO_3)$	$(Cu(CO_3)_2^{=})$	(CuL_{org})
Po A1	2.4×10^{-10} 0.5%	1.7×10^{-15} -	1.7×10^{9} 3.7%	2.1×10^{-11} 0.05%	4.4×10^{-8} 95.2%
Po C2	1.7×10^{-8} 3.7%	8.7×10^{-12} -	1.1×10^{-7} 24.7%	8.6×10^{-10} 0.2%	3.0×10^{-7} 66.2%
Po D1	3.0×10^{-10} 0.6%	2.9×10^{-15} -	2.8×10^{-9} 5.5%	3.3×10^{-11} 0.06%	4.7×10^{-8} 93.1%
Ticino	1.8×10^{-9} 4.5%	1.0×10^{-13} -	1.4×10^{-8} 34.6%	1.7×10^{-10} 0.4%	2.2×10^{-8} 56%

* concentration in mole/liter

** μmole/liter Cu

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Recent Advances in Electrochemical Techniques for Environmental Pollution Monitoring and Control

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ABSTRACT

In recent years there has been a real Renaissance in the theory and technology of electroanalytical chemistry. To a large degree this revival has been due to the unique applicability of these techniques for the analysis of trace levels of species of toxicological importance. Modern pulse and differential pulse polarographic techniques provide a relatively low cost approach to the trace analysis of a wide range of species including metals, organics, organometallics and pharmaceuticals of environmental significance.

The widespread interest in these techniques has resulted in developments in a number of areas.

i) The development of techniques such as stripping voltammetry for the determination of heavy metals, organometallics, and anions such as cyanide in natural waters and industrial effluent at the sub-part per billion level.

ii) The design of selective voltammetric detectors for monitoring organics either directly or following HPLC separation.

iii) The design of novel techniques such as hydrodynamic voltammetry which provide simple, low cost instrumental methods for pollution monitoring.

iv) The use of electrochemical principles for the development of electrochemical reactors for environmental pollution control. Advances in electrode design and hydrodynamic mass transport theory have resulted in the development of electrochemical reactor systems capable of removing heavy metals, cyanides and organic species from a range of process streams with a high degree of efficiency.

Keywords: Electroanalysis, pollution monitoring, polarography, voltammetry, hydrodynamic, electrochemical reactors.

The topic of environmental pollution is an extremely emotive one and frequently the target for misinformed publicity. Nevertheless, it is a fact that the continued expansion of industrial and urban society must require significant reductions in the amounts of waste pollutants now being discarded. The assimilative ability of the industrial countries air, water and land resources is rapidly approaching the maximum and further expansion or even operation at the existing level must result in severe health and social degredation.

An efficient environmental pollution monitoring system is an integral part of modern industrial society. It is necessary first of all to allow the various regulatory authorities to check compliance of industry with offical effluent limits and also to allow industry itself to pinpoint malfunctions and allow immediate correction.

The present review surveys the current status of electrochemical sensors, an area which is at present providing one of the most promising approaches to pollution monitoring. This review has attempted to cover three areas, firstly a survey of electroanalytical techniques and a comparison with competitive instrumental techniques in terms of selectivity, sensitivity and economics. Secondly the main types of pollutant species, toxicity and permissible regulatory levels are briefly surveyed and finally some new approaches to the problem of effluent control, based on electrochemical reactor systems are outlined.

In the last 10-15 years there has been a real revival of interest in electroanalytical chemistry (Flato 1973, Fleet and Jee, 1976). This is evidenced by the large number of new application areas some of which are highlighted below:

Renaissance of Electroanalytical Chemistry

* Development of high sensitivity, low cost instrumentation for ultratrace analyses such as pulse polarographic techniques
* Introduction of selective electrode systems - potentiometric types for ions e.g. K^+, Ca^{2+}, NO_3^-, etc. and diffusion electrodes (voltammetric and potentiometric types) for gases, organics and enzymes
* Ability to tailor electrode or electrochemical system for a wide range of species, cations, anions, gases, organics, organometallics, enzymes
* Unique application areas - toxic heavy metals, cyanides, drugs
* Biomedical applications both in-vivo and in-vitro clinical screening
* Involvement of electrode reactions in key problem areas of modern society including
  - -environmental pollution, electrodes used both for monitoring and control via electrochemical reactors
  - -energy conservation, battery and fuel cell research
  - -conservation of natural resources- use of electrochemical engineering to provide alternative synthesis routes for process chemicals
  - -medical research - electrodes for monitoring in intensive care situations, also fringe areas such as electrochemical bone grafting and acupuncture.

It is possible to trace the real origins of this revival to two significant events; the introduction of the operational amplifier in the mid 1950's provided the impetus for the rapid advances in instrument design and led to the development of modern variants of classical polarography, especially the more sensitive pulse techniques. The other significant development was the realisation that the glass electrode operated by an ion-exchange process which led to the development of the whole field of ion-selective electrode technology.

Nowadays there are an extremely large number of different electro-analytical techniques but from a practical viewpoint and also from space limitations only the more useful methods will be described here. A simple classification of electrochemical methods is based on the current flow through the working electrode. In one important group of voltammetric methods the finite current flow through the electrode is the measured parameter. This group comprises the techniques of polarography, voltammetry and various coulometric procedures. The second main group includes voltammetric methods with zero current flow (potentiometry) and mainly comprises pH measurements and more recently the rapidly expanding area of ion-selective electrodes (Buck, 1976). This topic has been reviewed in this volume by Thomas.

Modern Polarographic Methods

The classical d.c. polarographic method (Heyrovsky, 1923) involves the application of a d.c. voltage to a dropping mercury electrode and plotting the current as a function of voltage. The resulting plot is known as a polarogram. While the d.c. method is extremely useful for defining the electrode mechanism and also the optimum analytical procedure for the polarographic analysis of various pollutant species, it does suffer from a serious fundamental limitation in terms of sensitivity. When the electrode is polarised to a negative potential a layer of cations is attracted to the mercury-solution interface. This so-called double layer acts in an analogous fashion to an electrolytic capacitor and requires a finite current to charge it to the required working potential. This background charging current cannot be simply subtracted and seriously limits the sensitivity of the technique.

There have been a variety of approaches to solving this problem (Fleet and Jee, 1976) but only three methods will be discussed here. The first two are the most widely used techniques in analytical voltammetry while the third provides the basis for on-line analysis using electro-chemical sensors.

Pulse Techniques

One important group of polarographic techniques are based on the fact that the faradaic and capacitative (charging) currents show markedly differing time dependencies. By sampling the current for a short time interval close to the end of the drop life a significant improvement in sensitivity can be achieved. Although a number of techniques have adopted this principle only one or two have found routine acceptance. Pulse polarography was invented by Barker and co-workers at Harwell in the late 1950s (Barker and Gardner, 1960). In Barker's original technique a series of increasing amplitude voltage pulses were imposed on successive drops; the current was sampled at the initial potential and after a selected time following the pulse application. Subsequently various modifications to the

original technique have been made and nowadays pulse techniques are the most widely used in analytical polarography/voltammetry.

Utilizing the differing time dependences of faradaic and charging current pulse method imposes a series of pulses of increasing amplitude to successive drops at a preselected time near the end of each drop lifetime. The input signal waveform and current response obtained are shown in Fig. 1. The initially high charging current decays away very rapidly and the residual faradaic current is sampled during the final part of the 50-60 millisecond time pulse.

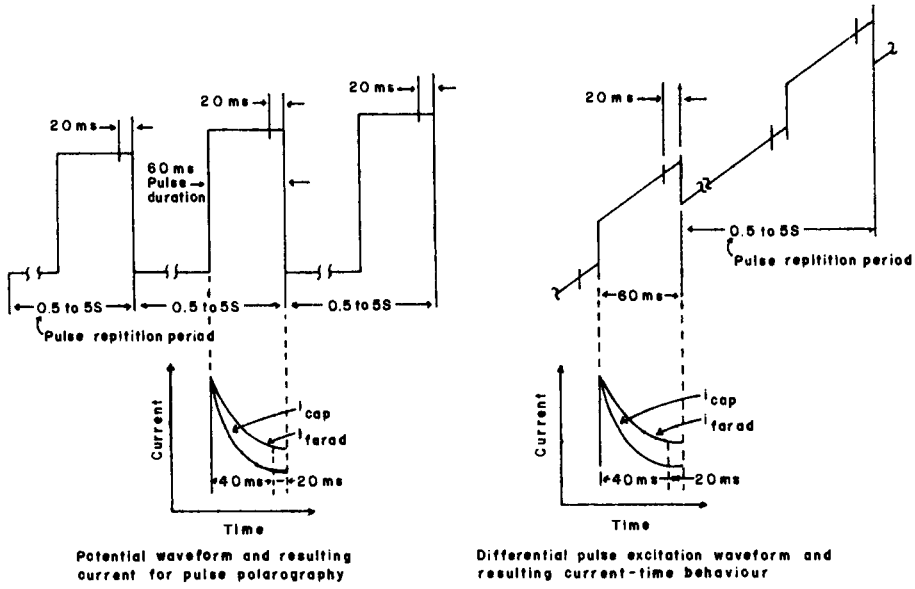

Fig. 1. Excitation wave form and current-time behaviour for a) normal pulse and b) differential pulse polarography

It is interesting to compare the diffusion limited currents for pulse and d.c. polarography as follows:

$$\frac{i_{pulse}}{i_{d.c.}} = \left(\frac{3t_p}{7t_d}\right)^{\frac{1}{2}} = 5\text{-}10$$

where t_p is the time after application of the pulse and t_d is the drop time in the d.c. mode. While this ratio predicts that normal pulse will only be a factor of 5-10 more sensitive than d.c., in practice the ratio is nearly two orders of magnitude. The additional increase in sensitivity is due to the ability of the technique to discriminate against the capacitance component.

Pulse polarography is extremely useful in analytical applications since it can respond to both reversible and irreversible processes. A further feature of the pulse technique is that as the measurement pulse is only applied for a small fraction of the total drop (0.5-5 seconds) only a small

amount of material is deposited on the electrode. One implication of this is that the pulse method is far less affected by problems of adsorption than in the d.c. technique.

Differential Pulse Polarography

While normal pulse polarography gives a marked improvement in sensitivity over the d.c. method, it still gives a similar S shaped polarogram. A much more useful variant, which is nowadays by far the most widely used polarographic technique for analysis, is differential pulse polarography. In this mode, small amplitude (10-100 mV) pulse of ca 60 m sec duration, superimposed on a conventional d.c. ramp voltage, are applied to the d.m.e. near to the end of the drop lifetime.

The current output is sampled at two time intervals (Fig. 1): immediately on the ramp prior to the imposition of the pulse and then again at the end of the pulse for ca 20 milliseconds when the capacitance current has decayed, and the difference in these two currents is then displayed. As the greatest increase in current for a given voltage increment will occur at the half-wave potential the i-E curve in d.p.p. will have a peak shape.

The theoretical relationship between the peak current, i_p, and pulse modulation amplitude, ΔE, has been derived by Parry and Osteryoung (1965). The maximum peak current when the pulse modulation amplitude is less than the value of RT/nF is defined as:

$$i_p = \frac{n^2F^2AC_o}{4RT}\left(\frac{D}{\pi t}\right)^{\frac{1}{2}}\Delta E$$

For a differential pulse polarogram with very small values of ΔE then the peak current potential coincides with the half-wave potential, $E_{\frac{1}{2}}$. With larger values of modulation amplitude, however the peak current potential is no longer coincident with $E_{\frac{1}{2}}$ and is given by

$$E_p = E_{\frac{1}{2}} - \Delta E/2$$

Consequently it is clear that maximum sensitivity in differential pulse polarography is obtainable for large values of ΔE. Increases in ΔE, however, also result in increased peak broadening with consequent loss of resolution and in general pulse amplitudes of 25-100 mV are mostly used.

Differential pulse polarography has found application to the analysis of a wide range of species of toxicological importance ranging from heavy metals to organics and organometallics (Smyth 1979).

Stripping Voltammetry

Inherently one of the most sensitive analytical techniques, stripping voltammetry (s.v.) has received a great deal of attention in recent years (Barendrecht, 1967; Vydra et al., Kissinger, 1976; PAR, 1974) especially

for environmental monitoring. It is extremely simple in concept, the method consisting of a two stage process; the first, a pre-concentration step, consists of controlled deposition of the species of interest onto a stationary electrode. This is followed by the measurement step which consists of electrolytically stripping the deposited species back into solution. In practical terms the pre-electrolysis step involves either the use of a stirred solution or a rotated electrode under carefully controlled hydrodynamic and electrolytic conditions while the stripping step involves the imposition of a potential step or any of the conventional polarographic input waveforms such as pulse, a.c. or voltage ramp. The magnitude of the stripping signal can be related to the concentration of the species of interest.

The pre-electrolysis step. A variety of techniques have been employed for the deposition step. The most popular has been based on the use of a stationary electrode in a stirred solution although rotated electrodes, continuous flow tubular or wall-jet electrodes, packed particle bed electrodes and several other designs have been described.

For deposition with a stationary electrode in a stirred solution it is essential that the hydrodynamic parameters be rigidly controlled. In practice this means a controlled stirring rate, electrolysis time and reproducible location of the working electrode in the electrolysis cell. Mathematical treatment has shown that the mercury film electrode is capable of more sensitive measurements than the hanging mercury drop electrode (h.m.d.e.) under a given set of conditions. In fact, it is recommended that for trace analysis at levels down to ca 1-10 pbb the h.m.d.e. be used and that the mercury film electrode is used for levels below this.

The stripping step. After pre-electrolysis the accumulated metal must be removed from the electrode in the stripping step. A variety of electrochemical and chemical procedures have been used for this step. The most common has been the use of a linear voltage sweep, i.e. the technique of anodic stripping voltammetry (a.s.v.) generally refers to stripping with a fast (ca 100-200 mVs^{-1}) voltage sweep from a cathodic to an anodic potential. More recently the differential pulse technique has become more popular for the stripping process since it offers a much more effective discrimination against the background charging current and hence an increased sensitivity (Copeland et al., 1973).

Analytical applications of anodic stripping voltammetry (a.s.v.). The main application of stripping voltammetry to date has been in the anodic stripping of trace metals, particularly toxic heavy metals such as lead, cadmium, zinc and mercury in a variety of sample media from natural waters to biological fluids such as whole blood (Siegerman and O'Dom 1972). An extensive bibliography can be found in Vydra et al. (1976).

The technique can also be applied to stripping of organometallics of free radicals, e.g. triphenyltin compounds which form radicals which can be stabilized by adsorption on mercury (Booth and Fleet, 1970a,b).

Cathodic stripping. This is a technique where the pre-electrolysis is carried out at anodic potentials and the stripping step consists of a cathodic voltage scan. This method has been applied to the determination

of halides and anions, (Kemula and Kublik 1963), organics which form insoluble mercury derivatives such as the dithiocarbamate pesticides (Brand and Fleet, 1967, 1968) and ore recently to a range of organosulphur drugs. (Davidson and Smyth, 1977). Metal ions such as Mn^{2+} and Pb^{2+} can also be determined via cathodic stripping of the respective oxides on either a carbon paste or rotating platinum electrode (Hrabankova et al., 1965; Monnien and Zinke, 1968).

Hydrodynamic Voltammetry

Techniques in which the working electrode operates in a stirred or flowing solution where mass transport is due to both diffusion and convection are becoming increasingly important. Originally as in the case of the rotating disc electrode the method was used for mechanistic studies where the controlled mass transport conditions enabled the study of rates of electron transfer or intermediate chemical steps in the electrode process to be made. More recently the theoretical basis of this technique has assumed considerable importance due to the increasing applications of voltammetric detectors in continuous monitoring applications in flowing streams. A useful concept for understanding the contribution of convection to the overall electrode process is that the Nernst diffusion layer (Fig. 2), which assumes that even under rapidly stirred conditions there is still a thin motionless layer (ca 0.1 mm) next to the electrode across which mass transfer takes place solely by diffusion. Further

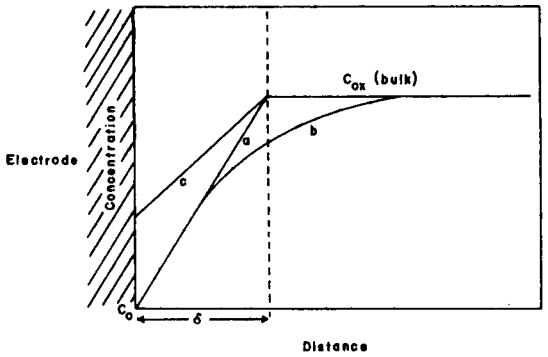

Fig. 2. Nernst diffusion layer, δ = diffusion layer thickness, concentration-distance profile under limiting current conditions (a) theoretical and (b) observed. Curve C shows concentration-distance profile at potentials below the limiting current on the ascending portion of the d.c. wave.

refinements in the theoretical treatment of hydrodynamic voltammetry with various electrode configurations have been carried out by Levich (1962) and co-workers while the principles of current distribution and mass transport in flowing systems have been reviewed by Newman (1973).

Continuous flow sensors. The increasing trend towards automation has been reflected in the wide range of electrode designs which have been described for continuous flow voltammetry (Tenygl 1979). These devices may be voltammetric in origin, for example, tubular electrodes, wire or

point designs, or thin layer cells. Alternatively an equally wide range of devices operating on coulometric principles have been reported. In these the electrode usually consists of a packed bed of conducting particles or fibres: in some cases porous electrodes (e.g. fuel cells) have been used (Fleet et al., 1972a,b).

Two designs of flow cell are worth noting. Tubular electrode configurations have been extensively studied by Olsen and Oesterling (1967a,b) and by Blaedel and Boyer (1971). These authors have also used a tubular mercury electrode with a platinum tube as the substrate for the mercury film.

Another promising design developed in the authors' laboratory, is the wall-jet cell (Fleet and Little, 1974; Fleet et al., 1978). This device is a thin layer cell where the sample solution enters the cell through a fine nozzle and impinges on the centre of a planar glassy carbon disc electrode. Reference and auxiliary electrodes are located downstream of the working electrode. The features of this cell design are the high sensitivity due to enhanced mass transport at the point of solution impact, ultra-low dead volume of the order of 0.5-5 µl, and the wide operating potential range of the glassy carbon electrode. It has found application for on-line monitoring of metals, continuous flow anodic stripping voltammetry and as a detector for high performance liquid chromatography (EDT Research, 1976).

A schematic diagram for the experimental assembly for automated anodic stripping voltammetry is shown below:

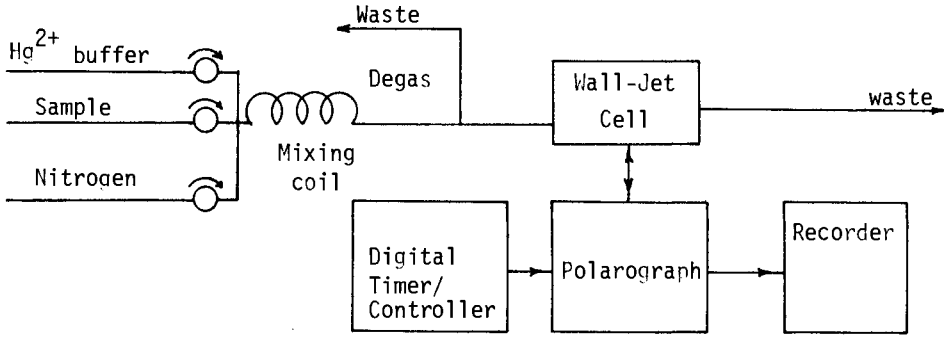

Figure 3. Automated Anodic Stripping System using the Wall-Jet Cell.

In this procedure the co-deposition technique of Florence (1970) is used where Hg^{2+} is added to the background electrolyte and the mercury film and the trace metal of interest deposited simultaneously on the glassy carbon electrode of the Wall-Jet Cell. Due to the enhanced mass transport the time required for the pre-electrolysis step is considerably reduced. Thus analysis of copper and zinc at the 1-10 ppb level can be carried out with a total analysis time of 90 seconds. (Fleet et al. Analyst 1979), giving an almost real time measurement of toxic heavy metal levels.

THE PROBLEM

The range and complexity of pollutant species continues to expand at an alarming rate and the updating list of toxic materials, commonly referred to as the 'Cancer of the Month' club provides a constant challenge for the ingenuity of the analyst.

The United Nations Environment Program, following its conference in 1972 instituted a Global Environment Monitoring System (GEMS) which has established a priority list of pollutants which includes heavy metals and chlorinated hydrocarbons amongst its key categories.

The real situation is rather less clear. In the United States the Environmental Protection Agency issues guidelines which aim eventually to be transferred into law. The aim of the EPA is to derive standards which take into account a thorough and sophisticated understanding of the effects of a wide range of potential toxic substances on public health, ecology and the economy. The technology standards applied are generally based on the demonstrated capability of technology to control pollution rather than a detailed understanding of the toxic effects of a given pollutant.

Analytical methods for measuring the large number of priority pollutants are clearly a prerequisite both for obtaining an adequate understanding of their environmental and health impacts. Clearly, also in order to apply any effective regulatory controls the sensitivity and limits of detection of the various measurement techniques must be precisely known.

Measurement techniques used for enforcement must be relatively simple, and inexpensive since these are performed in thousands of control laboratories and plants throughout the country. Recommended methods and guidelines are invariably governed by the availability of the methods so that any improvement in technique often results in a lowering of the legal limit.

Problems arise when industry is unable to meet these guidelines so that lengthy litigation suits are invariably in progress. Further confusion arises due to the complex socio-economic factors involved in enforcing these guidelines and only recently the White House issued a directive that enforcement of reduced pollution levels is inflationary. Yet another source of confusion is caused by the multi-tiered levels of regulatory bodies so that it may happen that the local State authority can attempt to enforce lower levels than the EPA guidelines.

Whatever the outcome of this situation it seems realistic to assume that regulatory levels must always be governed by the available analytical procedures.

THE SOLUTION

The wide range of conventional waste treatment technologies are mainly based on physico-chemical methods. Most widely used is chemical treatment either as hydroxide precipitation of metal ions, chlorination of cyanide containing wastes, etc. Additional methods include ion-exchange, evaporation, electrodialysis, reverse osmosis and adsorption methods.

With few isolated exceptions these methods are failing to meet the increasingly severe restrictions on permissible effluent levels. Conventional chemical treatment of metal bearing effluent via chemical precipitation also presents real problems due partly to the large size of the plant required and secondly the system produces a metallic pollutant in the form of sludge. In the U.S. controls on land fill dumping of sludges has become severely controlled with the realisation of the potential hazards these materials present due to rain leaching into natural water courses.

Electrochemical Reactors for Pollution Control

Electrochemical reactor systems provide the most promising new approach to pollution control. A variety of electrochemical systems have been employed but due to unsatisfactory design have not been able to handle large volumes efficiently and economically.

Recent advances in reactor design, most notably at HSA Reactors Limited, have dramatically altered this situation and whole new horizons for pollution control technology have now been opened. Electrochemical systems offer many attractive and unique features in the area of pollution control. They are inherently 'clean' systems with the only reagent,the electron,producing no unwanted waste products. Also they can operate in a resource recycling mode with costly process chemicals being recycled to the process plant.

Electrochemical Reactor Design

Due to limitations of space only a brief overview of the principles of reactor design can be given here. More detailed discussion of the problems are presented elsewhere (Newman 1973, Das Gupta and Fleet 1979). The existing designs for electrochemical reactors fall into two categories.

i) Two-dimensional reactors - typically the plate type cells used in classical electrowinning cells, and

ii) Three-dimensional reactors - based on packed beds of electrode material either metal or metal coated glass spheres, etc.

The primary design requirements in electrochemical reactors are high mass transfer rates while simultaneously having a controllable and uniform electrode potential over the entire electrode surface. Attempts to meet these requirements have not met with much success. In one approach the working electrode consists of a bed of small metal or metal coated glass spheres. The process solution is then forced vertically through this 'fluidised' electrode bed which is in electrical contact with a feeder plate or diaphragm. The main limitation to this design is due to the fact that the solution layer surrounding each particle causes an ohmic potential drop so that uniformity of potential throughout the bed is impossible to achieve.

HSA Electrochemical Reactor

The reactor design developed by HSA (Fleet and Das Gupta, 1976, 1977) uses a carbon fibre based composite electrode material which exhibits an extremely high surface area with uniform potential throughout the bed. The enhancement in mass-transfer characteristics exhibited by this design is best illustrated by the concept of space time yield; this parameter defines the efficiency of the electrochemical process, in terms of the electrode/cell volume. The HSA reactor shows space time yields in excess of one thousand time greater than the best competing fluidised bed system. Several studies at laboratory and pilot plant scale have optimised the reactor design for a number of pollution control areas.

Application of the HSA Reactor System

Some applications of the application of the HSA Reactor System (Kennedy and Das Gupta, 1978) include the reduction of metal ions such as the removal of toxic heavy metals, mercury, cadmium, lead, zinc and copper from electroplating and industrial effluent streams and subsequent recovery of the metals. The recovery of metals such as nickel and hexavalent chromium from plating liquors is another example and also the reduction of dilute precious metal streams such as in the gold cyanidation process. It is also possible to carry out anodic oxidation processes in particular the electrooxidation and electrochlorination of cyanide ion, the oxidation of phenols and various organic effluents including Kraft liquor from paper pulp manufacture.

Conclusions

Electroanalytical chemistry, which in its broadest sense can be defined as the study of electrode processes is a rapidly expanding field. Due to its key involvement in many of the problem areas of modern society it has seen an enormous revival of interest.

The development of new electrochemical monitoring techniques is a fruitful area and with the advent of the microprocessor the era of dedicated multiparameter in-line monitoring systems cannot be far away. One interesting development here is the ion-sensing transistor (Zemel, 1975) where the metal gate on a conventional metal oxide field effect transistor is etched away and replaced with a layer of ion-sensing ligand. The main attraction of this idea is that on a typical MOSFET chip measuring ca 1 cm^2 it is possible to have 50 gates - or consequently 50 individual sensors.

Electrochemical reactors which depend basically on the study of electrode processes show an enormous potential for environmental pollution control and, in the authors opinion, will most probably provide the dominant technology in this field. In short, electrochemistry should prove to be the solution to the world's problems!

Acknowledgements

I would like finally to acknowledge the contribution of my various colleagues and co-workers who, over the years have collaborated and stimulated my interest in this field. These include Dr. Chris Little, Dr. Tony Ho, Dr. Hari Gunasingham, Dr. Tom Ryan, Dr. Terry Berger, Mr. Bill Farrell, Dr. Graciella de Damia, Dr. Sankar Das Gupta, Mr. Ian Kennedy and Miss Julie Sharma.

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Applications of Chemiluminescence to the Metal Ion Determination in Water

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ABSTRACT

The chemiluminescence reaction method is applied for the determination of Cu^{2+} and Pb^{2+} ions in water. The light emission produced from luminol reaction in aqueous solution in the presence of hydrogen peroxide is used. The influence of metal ion solutions (10^{-3}M to 10^{-12} M) on this chemiluminescence emission is studied. The linear calibration curves for Cu^{2+} (10^{-5}M to 10^{-10} M) and Pb^{2+} (10^{-5}M to 10^{-7}M) are given. A simple detection system employing silicon photodetectors is suggested.

KEY WORDS: Chemiluminescence, trace metals (Cu^{2+}, Pb^{2+}), luminol.

INTRODUCTION

Chemiluminescence arises when a chemical reaction produces an electronically excited state in a molecule which emits light as it returns to its ground state. The development of sensitive detectors (photomultipliers) has made it possible to follow very weak luminescence, opening the possibity to rapid, highly sensitive analyses by chemiluminescence using inexpensive instrumentation.

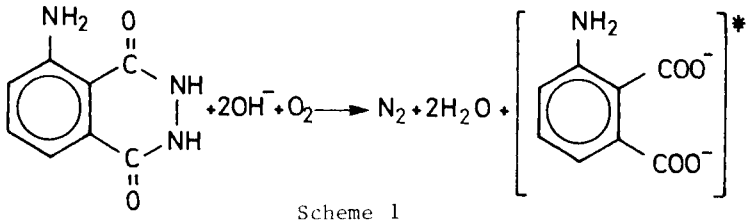
In particular, the luminol (5-amino-2, 3-dihydro, 4-phthalazinedione) emits intense chemiluminescent light in alkaline medium, in presence of oxidizing agents (e.g., hydrogen peroxide). This reaction is catalysed by metal ions. In presence of excess reagents, the intensity of light emission is proportional to metal catalyst concentration, a property that can be applied for metal ions determination. All methods reported for metal ion determination are based on catalysis of the luminol reaction, and most of them use photographic detection (a review article: Isacsson and Wettermark, 1974).

In this work, the kinetic chemiluminescence of luminol reaction in presence of Cu^{2+} and Pb^{2+} in aqueous solution is studied using photoelectric detection. The calibration curves for metal ion concentration are obtained and the sensitivity of the method is shown.

For customary determination a very simple, inexpensive and not heavy detection system is suggested. Its advantages and limitations are presented.

THEORETICAL

There is not a clear understanding of why some reactions are chemiluminescent. Different mechanisms for the chemiluminescence of the luminol reaction have been reported (Albrecht, 1928; Qyickenden, 1964; White, 1961, and White and colleagues, 1964, a,b). We believe that the reaction is of first order in base, luminol, and oxygen (scheme 1). The latter is produced during the decomposition of hydrogen peroxide in alkaline medium. In this reaction the inactive dianion of 3-aminophthalic acid is obtained in an electronically excited state (Armstrong and Humphreys, 1965), yielding a blue light ($\lambda_M = 427$ nm, Fig. 1).



Scheme 1

This reaction can be actived by the presence of metal ions; in consequence the emission effect is increased or quenched (Fig. 2). The increasing or the quenching can be attrituted to chemical processes whose overall effects do not reflect the stoichiometric reactions (Lukovskaya, 1969). Only certain ranges of metal ion concentrations show a linear variation with the intensity of emitted light (really, the logarithm of peak height of kinetic chemiluminescence curves is proportional to logarithm of metal ion concetration, Figs. 3 and 4).

EXPERIMENTAL

Reagents and standard solutions

Luminol solution, .5 10^{-3}M (from luminol crystalline Sigma) in .02 N NaOH. About 10^{-3} M H_2O_2 (from perhydrol Merck) in 10^{-4} $K_3Fe(CN)_6$.

Copper and lead solutions, 10^{-3}M to 10^{-12} M, were prepared freshly by dilution of the 10^{-3}M standard solutions.

All the reagents used were of analytical purity. Demineralized distilled water was used to prepare standard and reagents solutions.

Procedure

The reactions were carried out in a fused silica spectrophotometric cell of 1.0 mm. optical path. The reagents were mixed in the sample cell, and 1 ml of luminol solution and .2 ml of standard ion (Cu^{2+} or Pb^{2+}) solutions added. The content were mixed by agitation and allowed to stand for 10-15 minutes. The chemiluminescent reaction was started by the addition of hydrogen peroxide solution (1 ml).

Instrumentation

The equipment used to obtain the emission spectra for the luminol reaction consists of a high intensity grating monochromator with mechanic scanner (Bausch & Lomb 33.86.25.07), an UV-Visible photomultiplier (Oriel 7060), a stabilized high voltage power supply (Keithley, 244), a current amplifier (Keithley, 427), and a strip chart recorder (Hewlett-Packard, 7044 A). The monochromator has been used with an aperture exit slit of 2.68 mm. (spectral resolution = 4 10^{-2}), the photomultiplier operated at 600 V, the amplifier worked at 10^{7} for the gain, and the recorder with a 5 V/cm scale.

The total light spectral emission was registered employing the photomultiplier and the chart recorder. A charge resistence (.1 MΩ) was required. The voltage applied across the photomultiplier was held at 400 V, and the recorder was used in a 25 mV/cm scale.

The cell and the detector head was mounted on a firmly fixed optical beanch, and the system placed in a light-free box. Reagents and samples can easily be exchanged through a lock.

RESULTS AND DISCUSSIONS

The intensity of chemiluminescence depends strongly on the luminol concentration in the reagent mixture. Also, there is variation of the light emission with hydrogen peroxide concentration and with the reaction cell pH (Seitz and others, 1972; Isacsson and Wettermark, 1976). The quantities given in the experimental reagents were regarded as optimal conditions.

Fig. 1 shows chemiluminescent emission spectra for the luminol reaction. The continuous curve is the registed experimental spectrum and the dashed curve is the corrected emission spectrum with a peak at 427 nm.

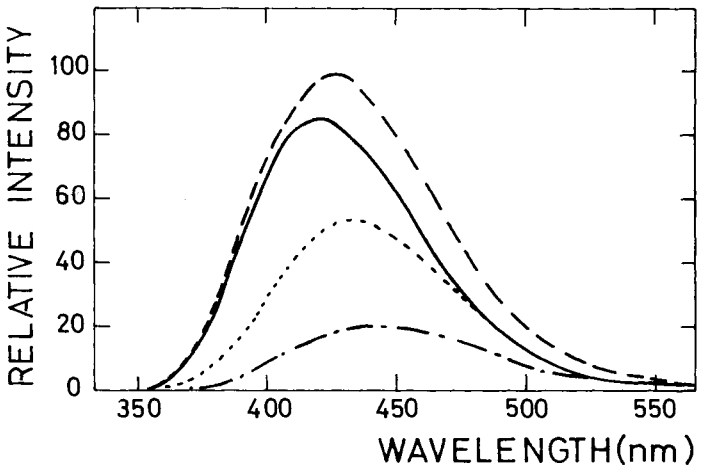


Fig. 1.- Luminescence spectrum from the luminol reaction: experimental (continous curve, Oriel 7060 photomultiplier) and corriged results (dashed curve).

The kinetic chemiluminescence curves were obtained from records of total light spectral emission. The total spectral emission detected by the photomultiplier is a .825 factor of the total spectral emission. Typical kinetic chemiluminescence curves are shown in Fig. 2. The effect of increased (I) or quenched (III) emission is shown by comparison with the kinetic chemiluminescence curve from luminol reaction (II).

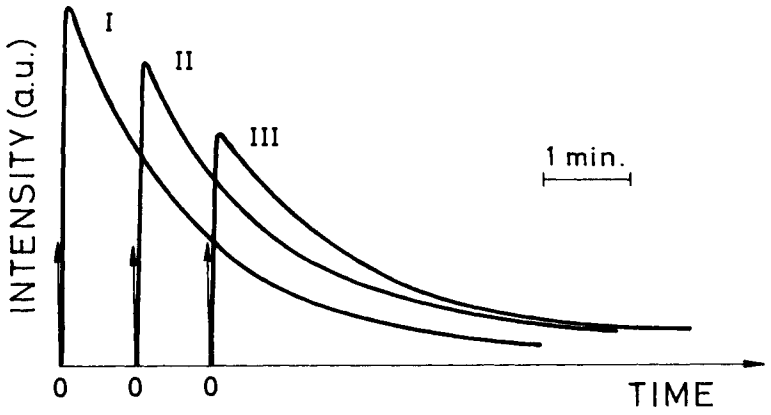


Fig. 2.- Kinetic chemiluminescence curves:
I) 10^{-6}M Cu^{2+} molar concentration.
II) Luminol reaction (reference).
III) 10^{-6}M Pb^{2+} molar concentration.

The peak height of kinetic curves derived from different values of ion concentration does not shown identical tendency for Cu^{2+} or Pb^{2+} ions. In the reactions with Pb^{2+} concentration, the peaks are lower than in the luminol reaction.

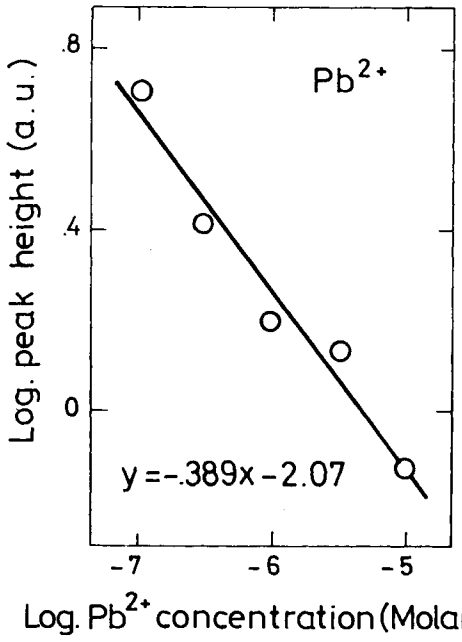


Fig. 3

The results show (Fig. 3) a linear calibration curve for the range 10^{-5}M to 10^{-7}M Pb^{2+} molar concentration using a double longarithmic plot. The adjusted linear equation is given.

In reactions with Cu^{2+} ions there are ranges where the peaks are lower than in the luminol reaction ($< 10^{-10}$ M, and $>10^{-4}$M). In the 10^{-5}M to 10^{-10} M Cu^{2+} concentration range the peaks are heigher than in the luminol reaction. In the latter range the peaks give to a linear calibration curve (Fig. 4). The regression line is given.

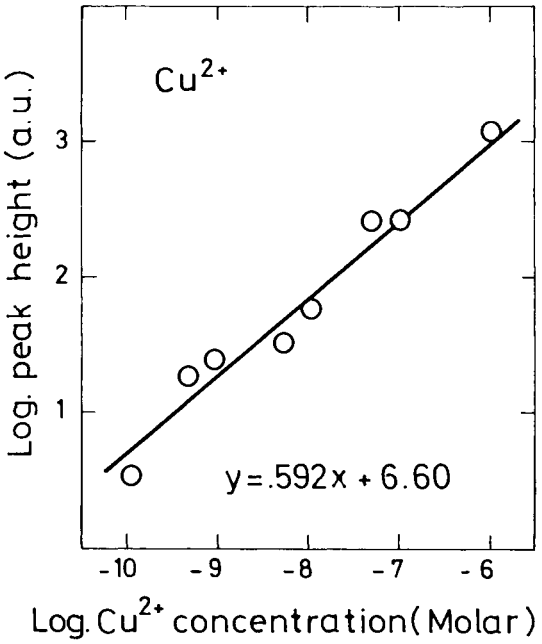


Fig. 4

The precision of the measurements has been estimated to be better 10%, but we found a greater stability and sensibility in the chemiluminescence reactions with Cu^{2+} ion concentrations.

Finally, two advantages of this analytical method are that no pretreatment of samples is required and the analysis time is short ($\lesssim$ 20 minutes).

A SIMPLE DETECTION SYSTEM

A comparative study of the most relevant characteristics (time resolution, linearity and the signal-to noise ratio) of general purpose photomultipliers (e.g. Oriel 7060) and silicon photodetectors (photodiodes and photovoltage detectors) shows the advantage of the silicon photodetectors for reducing cost, and obtaining a very simple and not heavy detection system. This applicability has been proved in resonant absorption spectrometry (Bernabeu, 1977). For their application in luminescence the silicon photodetectors of large area (e.g. Quantrad Corp.) are necessary. The spectral response of these detectors to blue and violet is low. Fig. 1 shows the relative sensibility for luminol chemiluminescence of photovoltaic detectors (PV-series^{+}, in dotted curve) and diffused junction photodiodes (LL-series^{+}, dashed - dotted). The to-

tal spectral sensitivity for these detectors is a factor .5 and .3 respectively of the sensitivity of photomultiplier.

Moreover, the responsitivity (photocurrent) of silicon photodetectors is lower ($\sim$ 500 for PV and $\sim$1200 for LL) than in the photomultiplier. This can be compensated, in part, using several large area photodetectors in parallel. Four photodetectors surrounding a square parallelepipedic cell is suggested (in particular, 30 x 10 - PV - CM and LL - 2E types[+]).

In order to prevent the noise in the detection, some thechnical features concerned with the coupling electronics are required (Bernabeu, 1977).

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Improvement of the Cold-vapor Atomic Absorption Method for the Determination of Mercury in Sea Water

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ABSTRACT

By proper optimization of the operating parameters, a noticeable increased sensitivity of this method is obtained. A combination of a preconcentration procedure (reduction-aeration process by trapping mercury on gold) and of the use of a small-sized cell for the final absorption measurement is proposed.

Sensitivity of 44 pg (mass Hg to 1 % absorption) is obtained. For comparative purposes, sensitivities of ≃ 100 ng with flameless atomic absorption (carbon rod atomizer) and of ≃ 5 ng with basic versions of the cold-vapor technique, are achieved.

The method was applied to the analysis of surface sea water samples from Ligurian Sea collected at two stations. From the first results (unfiltered and acidified samples) concentration range between 0.8 and 74.8 ng l^{-1}, median value 5.6 ng l^{-1} (n = 49) were reported. In these mediterranean marine waters, mercury levels did not differ significantly from those of other oceans and seas.

INTRODUCTION

For the routine determination of mercury in marine environment, especially in sea water where natural levels would not exceed 10 ng l^{-1}, investigation to increase the sensitivity of conventional method is needed yet.

Usually, analysis of mercury are conducted by cold-vapor and atomic absorption procedure (Hatch and Ott, 1968). With the two basic versions of the cold-vapor technique (closed and open systems), a typically 5 ng sensitivity (mass Hg to 1 % absorption) is obtained. Indeed, the measured maximum absorption is corresponding to 5 to 7 % of the total mercury released from the solution and circulating between the sample and the spectrophotometer cell. Then, if a preconcentration procedure is used, such as trapping mercury vapor with gold, silver, $KMnO_4$, activated carbon..., an increased sensitivity may be expected.

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ANALYTICAL PROCEDURE

A brief outline of the method and of the operating conditions is given in Fig. 1.

In a first step, involving reduction of a 50 ml acidified sample with stannous chloride, and aeration process by bubbling argon (900 ml min^{-1} gas rate) through the sample for 3 minutes, vaporized mercury is concentrated by trapping on gold (≃ 100 mg gold wire as pellet, packed in a thin quartz tube).

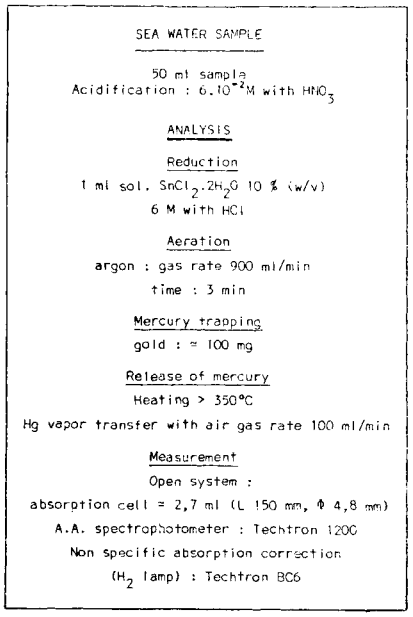

Fig. 1. Analytical procedure scheme

Then, in a second step, total mercury is easily again vaporized by heating gold trap (flame burner), and vapor is carried in a small volume of carrier gas (air gas rate ≃ 100 ml min^{-1}) through a relatively small-sized optical cell (≃ 2.7 ml) for the final absorption measurement (open system). A non specific absorption corrector is used. Peak height is measured and compared with a standard calibration line after blank determination. Schematic representation of the analytical system is described in Fig. 2.

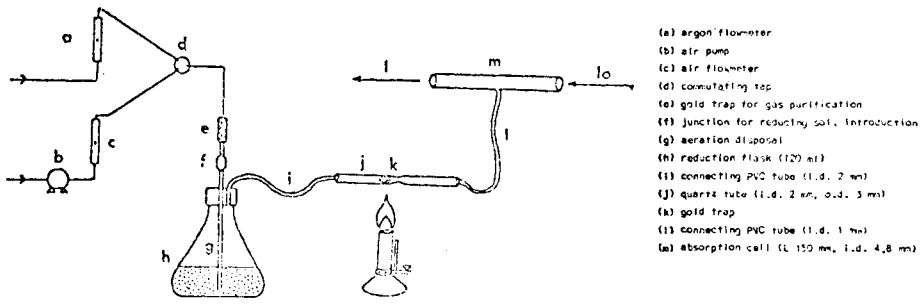

Fig. 2. Schematic representation of analytical system

The selected operating parameters result in an optimization of the method, with a sensitivity of 44 pg (mass Hg to 1 % absorption). Relatively to the commercially available apparatus with a detection limit of typically 100 ng l^{-1}, an increase factor of 100 is estimated. The precision expressed as variation coefficient % (CV %) is ≃ 5 % at the 10 ng l^{-1} range.

AERATION PROCEDURE AND TRANSFER OF MERCURY VAPOR FROM SOLUTE TO GOLD TRAP

Aeration of Hg enriched solution

Investigations are carried out on Hg enriched solutions to determine appropriate conditions for the quantitative aeration and transfer of metallic mercury released after reduction. Experimental results are reported in Table 1.

TABLE 1 Mercury vaporization : aeration time influence

T (sec.)	% Hg on gold trap	Aeration check 180 sec. (% Hg)
15	51.5	48.5
30	72.0	27.8
45	84.0	15.5
60	90.7	9.5
75	95.0	5.0
90	97.3	2.5
105	98.7	1.0

(900 ml min^{-1} argon gas rate)

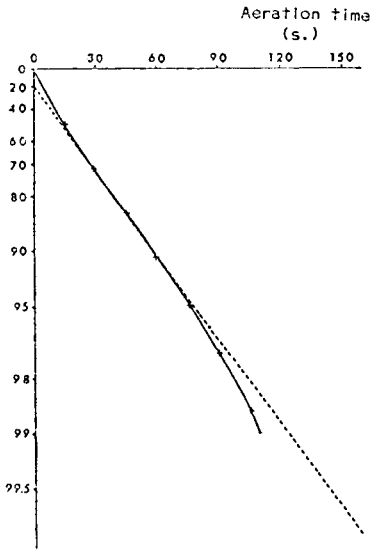

Fig. 3. Hg transferred on gold trap

From Fig. 3 where mercury trapped on gold (%) is represented on logarithmic scale, it is shown that the mercury concentration in the solution decreases exponentially as a function of the aeration time. Practically, a 3 minute aeration time reduces by a factor 10^3 the mercury concentration in the sample. Non linear deviations of the experimental curve can be explained : so, at zero time, linear extrapolation gives a 20 % value due to the metallic mercury initially degassed from the solute just as reducing agent and solution are mixed ; at the end curve, inaccuracy on the measurements are very amplified by the representation (theoretical linear curve is in the limit of 1 % uncertainty relative to the experimental curve).

Therefore, in a 50 ml sample at ambient temperature, quantitative (more than 99 % efficiency) vaporisation and transfer of mercury may be obtained after 3 minute aeration time with argon gas rate at 900 ml min^{-1}.

Aeration of C_6H_6 enriched solutions

To study aeration process, further investigations with C_6H_6 enriched solutions (50 ml sample with 400 mg l^{-1}) are also conducted. From aeration with various gas rates (from ≃ 200 to ≃ 900 ml min^{-1}), absorption at 253.7 nm is measured. Experimental results (Fig. 4, 5) show a quantitative vaporisation of C_6H_6 (after 4 to 20 minutes) depending on the gas rate.

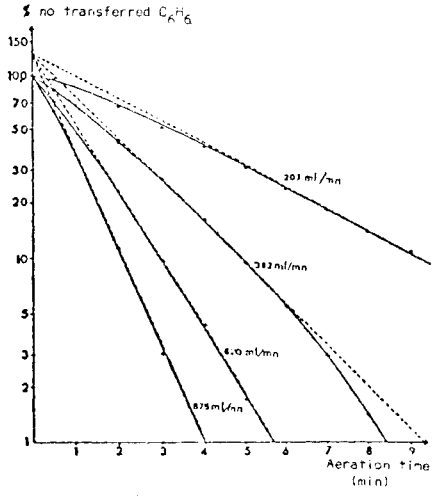

Fig. 4. Aeration of C_6H_6 enriched solutions

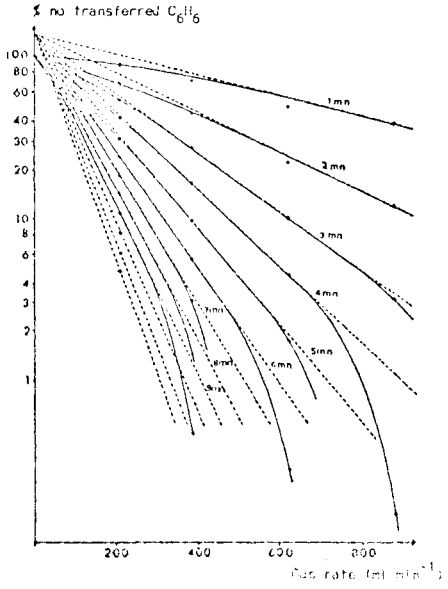

Fig. 5. Aeration of C_6H_6 enriched solutions

A linear relation between the vaporized C_6H_6 (transferred %) logarithm and the time or the gas rate is shown. That is significant of an exponential decrease of the compound in the solution, as mentionned above. Application of the exponential law demonstrates that it is the gas volume ("gas rate x time" product) running through the solution which affects the aeration yield, whatever time or gas rate conditions may be involved in it.

RELEASE OF MERCURY FROM GOLD AND MEASUREMENT IN ATOMIC ABSORPTION

After previous concentration, total mercury trapped on gold is very rapidly (typically in much less than 1 seconde) swept away by heating (flame burner). Due to the relatively small size of the trap resulting in reduced thermal inertia, gold may be heated at very fast rate resulting in increased analytical sensitivity : that does justify the use of the described above trap.

Using a small dead volume (here approximately 0.5 ml) between the trap and the measurement cell, allows the transfer of mercury vapor in a small carrier gas volume (estimated at ≃ 3 ml from experimental results, i.e., ≃ 6 times the dead volume) and so, limits the dilution effect for the mercury absorption measurement.

Mercury vapor carried through the optical cell gives an absorption peak which varies according to the gas rate : with air gas rates from 20 to 150 ml min^{-1}, recorded signals are presented in Fig. 6.

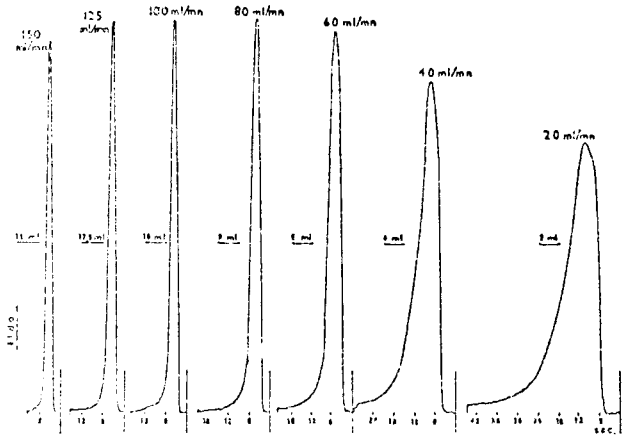

Fig. 6. Hg absorption peak with various air gas rates

Selected operating conditions are 100 ml min^{-1} gas rate, for peak height measurement.

In conjunction with mentionned above conditions, an adequate cylindric cell (L 150 mm, i.d. 4.8 mm) has been selected. Indeed, investigations with various lenght and diameter cells lead us to observe an important increase of sensitivity by reducing progressively diameter. Nevertheless, the total volume of the cell does exceed 2 ml (experimental data, depending on gas rate used and apparatus time constant for measurement).

Relatively to the spectrophotometer and the optical cell size used, limitation due to the loss of light by focusing optical beam (here, factor transmission 17 % instead of 80 % with usual ≈ 30 ml cell) must be considered. So, an adjustement between increasing sensitivity (diameter influence) and loss of light (focusing effect) was accomplished.

On the account, the selected optical cell tended to approach conditions as in a graphite furnace used in flameless atomic absorption.

With improved operating parameters, the ability to achieve easy, rapid, precise and high sensitivity analyses is succeeded.

MERCURY IN LIGURIAN SEA : CONCENTRATION IN SEA WATER

The method was applied to analysis of surface water samples collected in Ligurian Sea. We report here some initial findings of investigations carried out in two stations (nearshore and 10 km offshore Villefranche Bay). Unfiltered samples, collected with Niskin bottle, were stored in teflon stoppered Pyrex bottle, and were acidified (6.10^{-2}M) with nitric acid.

A histrogramm (Fig. 7) was prepared from the analytical results to indicate distribution pattern of mercury concentrations. From 49 samples, only three values more than 10 ng l^{-1} were observed (values more than three standard deviations from the mean).

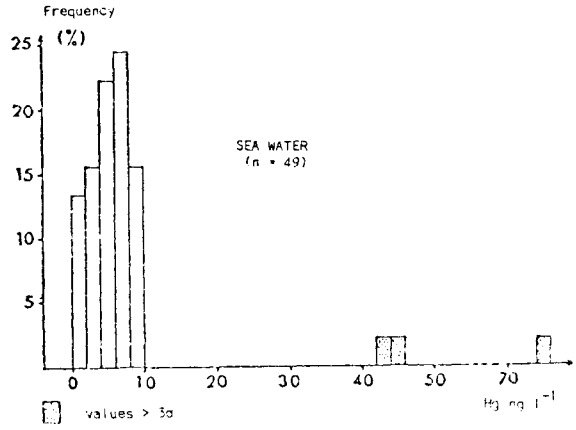

Fig. 7. Distribution frequency of mercury concentrations over the total water samples

The mercury concentrations range from 0.8 to 74.8 ng l^{-1} (average 8.2 ng l^{-1}, median value 5.6 ng l^{-1})(Table 2).

TABLE 2 Mercury values at oceanographic stations

Stations	Sample size	Range ng l^{-1}	Mean ng l^{-1}	SD	Relative SD %	Median value ng l^{-1}
Nearshore	26	1.4-42.4	6.8	7.7	113	5.6
10 km offshore	23	0.8-74.8	9.9	16.7	169	5.6
Total	49	0.8-74.8	8.2	12.7	155	5.6
	(46)	(0.8-9.6)	(5.2)	(2.6)	(50)	(5.4)

() Excluding high values (>3 σ)

The general amounts of mercury in the inshore waters are similar to those found in the offshore examined region. Mean concentration or median value presented in this Table are in good agreement with the overall range and possible baseline levels given by other workers (Baker, 1977 ; Burton, Leatherland, 1971 ; Fitzgerald, Lyons, 1975 ; Leatherland and others, 1971 ; Matsunaga, 1975, 1976) for Ocean waters throughout the world. Then the mercury levels reported seem to show that the

sampling area is not affected by any significant mercury pollution emphasis (Bernhard, 1978). Indeed, it seems that anthropogenic and natural mercury sources have only local influence, in Mediterranean sea water.

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Extraction of Heavy Metals from Marine Sediments for Analysis by Atomic Absorption Spectrometry - Some Factors Affecting Extraction Efficiency

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ABSTRACT

The acid extraction of heavy metals from marine sediments in closed vessels for subsequent analysis by atomic absorption spectrometry was investigated, with particular attention given to the effect on extraction efficiency of the composition of the acid mixture for sediments of different composition as well as amount of sample digested. It was found that the composition of the acid mixture used in the digestion has an important effect on the concentration of metals found. Using an acid mixture determined to be most efficient foravariety of sediment types, samples taken by grab and core samplers along the Turkish Eastern Mediterranean coast were analyzed for up to seven selected heavy metals.

KEY WORDS: Sediment analysis, heavy metals, acid extraction, atomic absorption.

INTRODUCTION

The analysis of marine sediments is an important area of environmental analytical chemistry. Heavy metals, in particular, have gained importance recently due to their known detrimental environmental effects. It is thus important to study the distribution of heavy metals in marine sediments, but for this a reliable analytical method is needed.

An important technique for the analysis of metals in a variety of environmental samples is atomic absorption spectrometry. For the analysis of solid samples such as sediment, a dissolution step must precede the analysis step as solutions are most easily analyzed by atomic absorption.

Solid samples are commonly digested with acid in open or closed vessels at room or elevated temperatures. The acid or acid mixture must be able to effectively decompose both the organic and siliceous material of the sediment or be able to efficiently extract the metals. For this purpose five mineral acids, viz., HCl, HNO_3, H_2SO_4, $HClO_4$ and particularly HF, have been used by many workers either singley or in a variety of combinations, employing open or closed digestion systems.

Bernas (1968) pioneered the bomb decomposition of solid geological samples using a mixture of aqua regia and HF to dissolve silicate materials. Omang and Paus suggested a mixture of nitric and hydrofluoric acids for the digestion of

geological materials in the bomb-type closed vessel (1971). Agemian and Chau demonstrated the advantages of digesting sediments in decomposition bombs when total extractable metal content, excluding mercury, is to be determined (1975). They recommended a mixture of $HF-HNO_3-HClO_4$. This mixture used with the bomb digestion technique was more efficient than any type of cold or hot extraction for a variety of different types of sediment. Other acid mixtures were not evaluated.

As the bomb technique is rapidly becoming widely-used for the decomposition and dissolution of various types of environmental samples, a study of the relative extraction efficiency of various acid mixtures with this technique, as well as other parameters affecting the digestion, would be valuable. In this study several commonly-used mineral acids and mixtures of the acids in various proportions were evaluated for their ability to extract several heavy metals from marine sediments ranging from sand to silt in nature and differing in their organic carbon contents.

Using the digestion conditions determined to be most efficient in general for a variety of different types of sediment, several samples of coastal and shelf sediments from the Mediterranean were analyzed for copper, iron, zinc, lead, manganese, chromium and nickel.

MATERIALS AND METHODS

Sample Collection and Preservation

Shore sediment samples were collected with a "VanVeen" type grab sampler. Continental shelf samples were collected with a "Shipek" grab sampler and a piston core sampler. The specimens were carefully removed from the inner portion of the samplers to avoid contamination; in the case of core samples, only the portion near the tip of the core was analyzed. All samples were sealed in plastic bags and stored at -30°.

Digestion of Samples

For analysis the wet samples were dried at 100°; the dried samples were then ground to a fine powder which was passed through a 70 mesh (0.2 mm) sieve. Digestions were carried out in PTFE-lined high pressure bomb-type decomposition vessels. About 50 mg of dried sample was digested with various acids at 140° for 4 hours. The digested samples were diluted to 50 ml with distilled-deionized water. When HF was used in the digestion mixture, 0.8 g boric acid per ml HF was added to the digested mixture to dissolve metal fluorides.

Analytical Procedures

The digested, diluted samples were analyzed by directly aspirating the solutions into an air-acetylene flame of the atomic absorption spectrophotometer. For zinc the burner head was rotated approximately 30° to the light path to reduce sensitivity. For iron, chromium and manganese less-sensitive absorption lines were used. The standard-addition method was used in all cases for calibration.

Organic Matter Determination

The organic matter content was determined by the chromic acid method (Olausson, 1975). To about 0.4 g of powdered sediment 10 ml of 1 N dichromate solution and 20 ml concentrated sulfuric acid are added carefully and the flask mixed gently for 1 minute. After 20-30 min. reaction period the solution is diluted to 200 ml with distilled water and 10 ml concentrated phosphoric acid. 0.2 g NaF and 1 ml diphenylamine indicator are added. The sample solution is then back titrated with 0.4 N ferrous ammonium sulfate.

RESULTS AND DISCUSSION

The five mineral acids in up to 15 different combinations were evaluated as extracting agents. In general it was observed that acids used singly were not as effective as mixtures. Sulfuric acid was a particularly poor extracting agent. Perchloric was the most efficient acid when used singly. To completely dissolve the sediments, HF was required. However, it was found that mixtures which did not contain HF, and thus did not result in complete sample dissolution were also effective.

In general it can be concluded that mixtures of HNO_3:$HClO_4$:HF are the best among all mixtures evaluated for a variety of sediment types. $HClO_4$: HCl mixtures which are also quite effective can be used when it is not desired to use HF due to the introduction of impurities from the boric acid treatment.

The importance of the acid used for digestion can be better understood from the data in Table 1 where the percent relative standard deviations among acids for three different types of sediment are presented. It is seen that for the sand and clay sediments, type of extracting agent used is quite important because of the wide variations observed among acids and mixtures. In the case of the muddy sediment, the choice of acid is less critical. These data demonstrate the importance of the nature of the sediment in determining the optimum extracting agent. The mechanism of incorporation of metals in sediments of differing chemical composition and physical character is obviously of prime importance, but is not well-understood and was beyond the scope of the present investigation.

When using the bomb-type closed vessels to digest sediment samples, the size of the sample digested may have an effect on the concentration of metal found, as seen in Table 2. The concentrations of nickel, chromium and lead appear to decrease with increasing weight of sample digested. The digestion period was kept constant at 4 hours for these experiments. A longer period would perhaps minimize the differences. 50 mg was chosen as the best compromise weight which allowed the metals to be detected by flame atomic absorption while at the same time leading to high extraction efficiencies.

With the optimum extraction conditions established, a study of the levels of some trace metals in a number of Mediterranean coastal and continental shelf sediments was carried out. The analytical results are presented in Tables 3 and 4.

TABLE 1. Relative Standard Deviations of Average Results of Three Types of Sediment Using Different Acid Mixtures

Sample	Organic Content(%)	Fe	Mn	Pb	Cu	Zn	Ni
Sand	1.03	15.9	22.4	-	-	34.1	-
Black mud	1.76	2.5	10.1	7.4	6.2	3.4	4.5
Clay	4.49	14.0	6.1	-	-	14.8	11.6

TABLE 2. Effect of Weight of Sample Used for Digestion on Concentration of Metal Found (μg/g).

Sample weight (g)	Fe	Mn	Zn	Cu	Cr	Pb	Ni
0.025	40185	413	587	149	628	418	391
0.050	39685	418	595	142	585	353	356
0.075	39835	422	560	146	512	345	327
0.100	40560	432	598	146	483	286	324
0.150	39270	427	586	153	479	271	318
0.200	38790	423	586	135	498	252	308
% Stand. dev.	1.6	1.6	2.3	4.3	11.6	19.4	7.1

TABLE 3. Average Trace Metal Concentration in Coastal Sediments (μg/g)

Location	Type	Fe	Mn	Zn	Cu	Pb	Cr	Ni
Ovacık	Sand	4247	170	16.9	-	-	-	-
	Sand	2987	135	18.9	-	-	-	-
	Sand	2761	115	16.5	-	-	-	-
Göksu	Sand	24460	434	48.9	21.1	130	-	169
Delta	Clay	29640	383	49.4	24.7	141	-	79
	Clay	33020	562	55.3	36.3	103	-	131
	Clay	30930	470	46.4	32.2	172	-	139
Limonlu	Mud	39700	787	62.8	32.5	53	594	556
	Mud	44400	715	74.5	33.3	55	535	480
	Mud	45100	742	72.7	37.2	63	534	477
	Sand	42700	784	47.4	23.7	-	-	332
Mersin	Mud	41730	411	483.0	368.0	280	-	294
Harbor	Mud	41610	415	406.0	112.0	155	-	325
	Mud	41100	612	69.0	25.0	93	-	343
	Mud	27320	389	44.0	24.0	46	-	104

TABLE 4. Trace Metal Concentration in Shelf Sediments (μg/g)

Type	Water depth(m)	Fe	Mn	Zn	Ni	Cu	Cr
Grab	380	39054	844	63.1	309	33.2	289
Corer	558 (3.1)#	38840	765	64.6	277	33.7	277
Grab	558	44634	1493	69.0	217	50.2	161
Corer	641 (2.4)	36853	819	81.4	247	33.1	225
Corer	450 (1.9)	38793	687	62.8	244	33.3	260
Grab	363	39216	717	75.6	277	22.5	298
Grab	1019	48180	1938	72.2	206	52.1	151
Corer	848 (1.7)	40541	990	63.5	332	29.9	305
Grab	151	42357	1499	75.4	264	42.3	246
Grab	294	41497	3335	71.8	239	46.8	198
Corer	198 (1.8)	42377	741	67.0	271	41.9	229
Corer	371 (3.0)	38198	857	54.2	319	34.5	344

Number in parenthesis indicates length of core

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Variations in Extraction Efficiency of Aqueous Cadmium (II) Using the APDC-MIBK Procedure

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ABSTRACT

The effect of pH and total cadmium (II) concentration on the efficiency of the APDC-MIBK chelation-extraction procedure was determined at an aqueous to organic volume ratio of 10:1. Efficiency determinations were based on the partitioning of an original aqueous sample activity of 1.75×10^5 dpm (as ^{109}Cd) between the organic and aqueous phases. Solution activities were determined by liquid scintillation spectrophotometry. The extraction efficiency was independent of pH in the range 2.0 to 9.0 at a cadmium concentration of 1.62×10^{-4} ppb radioisotope only. At pH values less than 2.0, the extraction efficiency dropped markedly and was 0.00% at pH 1.0. Alternatively, varying the concentration of cadmium in the aqueous solution (pH 2.8) led to high extraction efficiencies (>99%) for low concentrations (1-10 ppb) and low efficiencies (6-11%) for high concentrations (250-500 ppb). The effect of APDC:Cd molar ratio was also investigated at 1 ppb Cd. Extraction efficiency remained near 100% for APDC:Cd molar ratios of ≥14,000. Efficiency dropped to 43% at a ratio of 1,400, 3% at 140, and 0.00% at 14. Calculations show that the decrease in extraction efficiency with increase in Cd(II) concentration can be attributed to (1) insufficient APDC, and (2) saturation of the metal chelate in the organic phase.

INTRODUCTION

The chelation and subsequent extraction of trace metals is a method by which environmental analytical chemists can remove matrix interferences and/or measure metal concentrations below normal detection limits. Both situations can be encountered in the analysis of toxic metals in natural aqueous systems. Solvent extraction, i.e., the transfer of the trace metal of interest out of the aqueous medium into a less voluminous, immiscible organic phase, enables both absolute concentration and improvement of matrix character. It is a favored technique for environmental chemists because of its ease, speed, and simplicity. (Morrison and Freiser, 1967; Stary, 1964)

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A common extraction system utilized for atomic absorption analysis of trace metals employs chelation of the metal with ammonium pyrrolidine dithiocarbamate (APDC) and extraction into methyl isobutyl ketone (MIBK), a sparingly water soluble (1.9%) ketonic solvent (Brooks, Presley, and Kaplan, 1967). The APDC-MIBK procedure is used extensively for trace metals in fresh water (Fishman and Midgett, 1968; Paus, 1971), soil (Dudas, 1974), sediments (Agemian and Chau, 1976), sea water (Boyle and Edmond, 1975; 1977; Kremling and Petersen, 1974; Segar and Gonzalez, 1972), and biological samples (Hessel, 1968; Julshamn and Braekkan, 1975).

The ketonic solvent, MIBK, has stable flame characteristics and extracts well, coordination-unsaturated chelate compounds, i.e., those whose main valency bonds are saturated by the chelating agent but whose coordinate bonds are partly saturated by the organic agent and partly by water. The water solubility of the chelate is decreased and its organic solvent solubility increased by the displacement of the coordinated water molecules with the MIBK. The organic complexing agent, APDC, is used because of its chelating ability with a large number of metals (Malissa and Schöffman, 1955), most of which are quantitatively extractable over wide ranges (Allan, 1961; Mansell, 1965; Mulford, 1966; Pyle and Jacobs, 1964; Sprague and Slavin, 1964; Willis, 1962). Ionization in aqueous solutions of APDC releases the pyrrolidine dithiocarbamate anion (PDC) which acts as a bidentate ligand to form four-membered chelate rings with cadmium (Fig. 1).

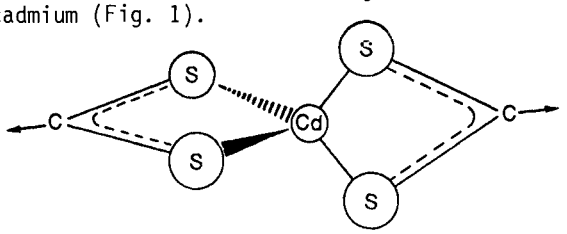

Fig. 1. Structure of $Cd(PDC)_2$ complex

The resulting dithiocarbamate chelate is particularly stable because of the contribution of resonance structure of the complex (Morrison and Freisner, 1957) and the availability of empty d orbitals on the sulfur to accept d electrons from the central metal in a π backbond (Burger, 1961). The dithiocarbamate ligands complex metal ions with partially filled d orbitals, ions with completely filled d orbitals but low positive charge, and ions with an 18 + 2 electronic structure (Hulanicki, 1967). Cadmium fulfils the last two criteria and therefore forms stable dithiocarbamate complexes. The presence of the pyrrolidine ring increases the organic solubility of the chelates over that of the dithiocarbamates with aliphatic nitrogen adducts (Morrison and Freisner, 1957). Four coordinate divalent cadmium possesses ten d orbital electrons and prefers tetrahedral geometry (Fig. 1), with sp^3 bonding (Cotton and Wilkinson, 1972).

The efficiency of an extraction system applied to an aqueous cationic metal depends on several factors including the chelating agent employed, the solvent system, concentrations of the metal ion and the chelating agent, pH of the aqueous phase, and interferences. Attempts in the author's laboratories to extract trace quantities of toxic metals yield, in some cases, erratic results and prompted research on the extraction efficiency of cadmium. Most of the published research on the APDC-MIBK procedure has been principally descriptive and operational. The least known aspect of the extraction method is its the-

ory. This investigation was conducted to determine the effects of (1) pH of the aqueous phase, and (2) total cadmium (II) concentration, on the extraction efficiency using the APDC-MIBK procedure. In addition, attempts were made to explain the theoretical aspects of the limitations of the procedure.

METHODS AND MATERIALS

Preparation of Solutions

Two hundred milliliter aliquots of distilled deionized water were used for all experiments. Aliquots for the pH experiments were prepared using the following pH values: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. Concentrated HCl or 1M NaOH was added in dropwise amounts until the proper pH value was obtained. The pH value after extraction was also determined and found to be within ±0.2 pH units of the value before extraction.

Metal concentrations were prepared at 1, 10, 50, 75, 100, 250, and 500 µg/l (ppb) Cd(II) from a stock solution containing 1,000 mg/l (ppm) Cd as $CdCl_2$. The stock and diluted metal standard solutions were maintained at pH 2.8.

The APDC:Cd molar ratio experiments were performed using a cadmium concentration of 1 ppb (at pH 2.8) and varying the concentration of APDC. APDC:Cd ratios of 14, 140, 1,400, 14,000, and 70,000 were used. The cadmium concentration, 1 ppb, was equivalent to 8.9×10^{-9}M. At the highest molar ratio (70,000), the concentration of APDC was approximately 0.1 g APDC/liter.

All experiments were conducted using the radioisotope, ^{109}Cd, which was chosen because of its relatively long half-life, 470 days, and decays via an electron capture energy of 0.16 MeV (also a gamma ray of 0.088 MeV). The electron capture mode of decay gives rise to conversion and Auger electrons which are efficiently detected in liquid scintillation systems (Peng, 1977). Counting efficiencies were typically in the 40% range for the aqueous samples. The organic phase showed some quenching; however, all sample activities were initially 1.75×10^5 disintegrations per minute (dpm)/200 ml aqueous providing sufficient activity to count. The counts per minute (cpm) were corrected to dpm using a pre-determined quench curve. An aqueous type scintillation cocktail, Aquasol (New England Nuclear), was used for the counting of both the aqueous and the organic phases. All samples were counted with a Beckman Liquid Scintillation Spectrophotometer. The use of radioisotopes for research in liquid-liquid extraction techniques is highly recommended. Measurements can be made directly, quickly, and accurately using either internal or external standization. Liquid scintillation techniques also permit accurate determination of very small metal concentrations in either of the liquid phases.

Extraction Procedure

A 200 ml aliquot of the solution to be extracted was transferred to a 250 ml separatory funnel and 1 ml of the 1.75×10^5 dpm/ml ^{109}Cd stock solution was added. The resulting cadmium concentration (from ^{109}Cd only) was 1.64×10^{-4} ppb. After mixing, 1 ml was removed to determine initial activity. The 1 ml was placed in a scintillation vial and 10 ml of Aquasol added. Initial aqueous solution activities were approximately 870 dpm/ml (1.75×10^5/201 ml).

Next, 1 ml of the 2% aqueous APDC solution (pH and metal concentration experiments only) was mixed with the 200 ml test solution. Ten ml of MIBK was pipetted into the separatory funnel which was then capped and shaken a few times

with periodic venting. The funnel was agitated on an automatic shaker table for 5 minutes after which it was allowed to stand for 30 minutes to permit phase separation. The aqueous phase was retained for a second extraction with the addition of 1 ml 2% APDC and 10 ml additional MIBK. The two MIBK extracts were added together (total volume 16.2 ml due to water solubility of MIBK) and 1 ml counted using Aquasol. The extracted aqueous phase was counted so that a total budget for the fate of the ^{109}Cd could be determined. The pH experiments were performed using only ^{109}Cd, while the metal concentration experiments necessitated the addition of non-radioactive Cd to achieve the desired metal concentrations. All tests were performed in triplicate.

RESULTS AND DISCUSSION

Effect of pH

The extraction efficiency (termed "recovery" by some researchers) is expressed as the percent of the radioactivity transferred from the original aqueous solution to the organic solvent, MIBK. It is derived simply by

$$\frac{\text{total activity of MIBK phase}}{\text{total activity of initial aqueous phase}} \times 100 = \text{extraction efficiency.}$$

The results of the extraction of the various pH adjusted solutions are shown in Fig. 2. The data show that the extraction efficiency is independent of pH in the range 2.0 to 9.0. Below 2.0, the efficiency drops rapidly (<10% at pH 1.5), and subsequently to 0.00% at pH 1.0. The principle of hard and soft

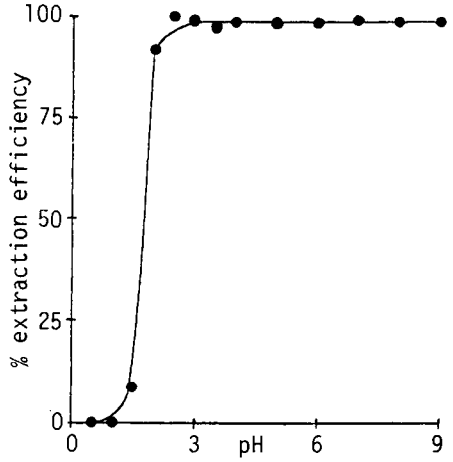

Fig. 2. Relationship between extraction efficiency and pH.

acids and bases (Pearson, 1968a; 1968b) helps to explain the results. Simply stated, the rule is that hard acids prefer to bind with hard bases and soft acids prefer soft bases. Cadmium (II) is a soft acid and RS^- is a soft base. The complex of a soft acid and a soft base, e.g., $Cd(PDC)_2$, tends to be quite stable especially in the presence of hard base (OH^-) and hard acid (H^+) and explains the wide pH range (2.0 to 9.0) over which the $Cd(PDC)_2$ complex is quantitatively extracted into MIBK. It would appear that below pH 2.0, the pyrrolidine dithiocarbamate anion is protonated and subsequently decomposed, probably to a protonated amine and carbon disulfide. The ligand degradation results in a reduction in extraction efficiency of aqueous Cd(II).

Effect of Initial Cd(II) Concentration

The results of the extraction of various cadmium concentration solutions are shown in Fig. 3. It is obvious that high efficiency, quantitative extraction occurs only at Cd(II) concentrations of 10 ppb and lower. At 50 ppb, the efficiency has dropped to a range of 80-85%. Dual data points indicate two sets of triplicates. The extraction efficiency drops quickly to 65-70% at 75 ppb, 21-24% at 100 ppb, 10-11% at 250 ppb, and 6% at 500 ppb. This decrease in

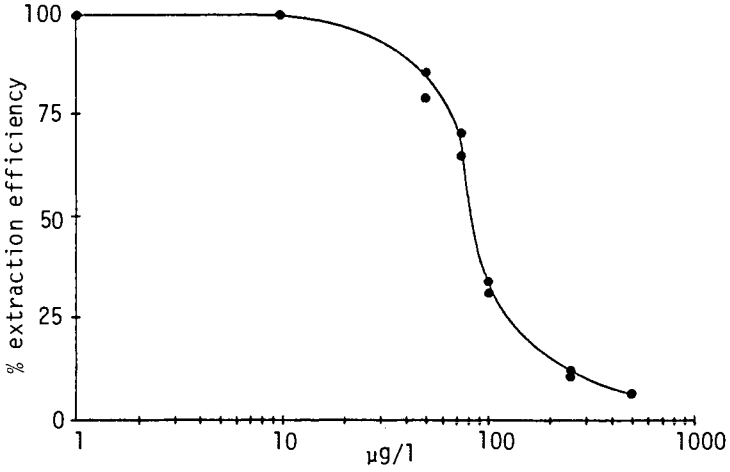

Fig. 3. Relationship between extraction efficiency and initial aqueous Cd(II) concentration.

extraction efficiency with increase in metal concentration could be the result of (1) insufficient APDC, (2) exceeding the solubility of the metal chelate in in the organic phase, or (3) the formation of a less-extractable polymeric form of the metal chelate. To elucidate (1), the effect of APDC:Cd molar ratio was investigated.

Effect of APDC:Cd molar ratio. Figure 4 shows the results of the variable APDC:Cd molar ratio experiments. For molar ratios of 14,000 and greater, the extraction efficiency of a 1 ppb Cd(II) solution was >99%. However, at an APDC:Cd ratio of 1,400, the recovery dropped to 43%. At a ratio of 140, the extraction was approximately 3%. These data imply that the amount of APDC in the solution is a critical factor. The data given in Table 1 substantiate the

Table 1 APDC:Cd Molar Ratios and Organic Phase Concentrations Versus Extraction Efficiency and Cd(II) Concontrations

Initial Aqueous Cd(II) conc. in ppb	Extraction Efficiency (from Fig. 3)	APDC:Cd Molar Ratio	Organic Phase in ppb
1	99%	140,000	12
10	99	14,000	117
50	81	2,800	467*
75	68	2,100	580*
100	31	1,400	369*
250	11	560	327*
500	6	280	369*

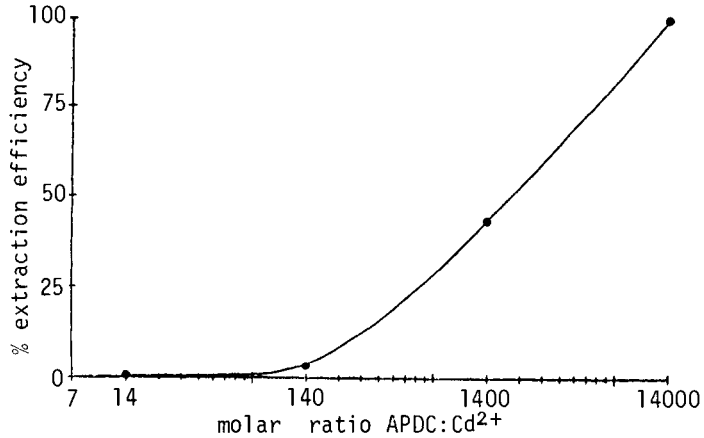

Fig. 4. Relationship between APDC:Cd molar ratio and extraction efficiency.

contention that a low APDC:Cd molar ratio was, at least, partially responsible for the depressed extraction efficiencies. These data are supported by the investigations of Kuwata, Hisatomi, and Hasegawa (1971) who reported a minimum sodium diethyldithiocarbamate:Cd ratio of 500 for quantitative extraction. Blanton (1974) showed that the APDC:Ni molar ratio extraction efficiencies varied with concentration. For example, at an APDC:Ni ratio of 700, the extraction efficiencies for 10, 50, and 100 ppb Ni were 89, 91, and 63%, respectively (Blanton, 1974).

Effect of metal chelate solubility. Blanton, Newland, and Ehlmann (1974) have shown that the solubility of the $Ni(PDC)_2$ complex in MIBK was principally responsible for the reduction in extraction efficiency with increasing Ni(II) concentrations. Similar calculations for the $Cd(PDC)_2$ solubility in MIBK are also shown in Table 1 (Organic Phase). The concentration of the $Cd(PDC)_2$ complex in the organic phase reaches a plateau as shown by those values with an *. The extraction efficiency begins dropping immediately. The "constancy" of the computed organic phase concentrations suggest saturation of this phase with the $Cd(PDC)_2$ complex. These data lend credibility to the importance of contention (2), i.e., exceeding the solubility of the metal chelate resulting in reduced extraction efficiencies. Hypothesis (3), i.e., formation of a less-extractable polymeric form of the chelate, is not likely since polymerization would have caused a change in coordination number. The higher coordination would probably have resulted in a color change for the complex and none was observed during the course of the experimentation.

CONCLUSIONS

With its 10 d electrons, cadmium is a soft central metal that forms strong bonds with the soft base, the pyrrolidine dithiocarbamate ligand. The strong complex results in high quantitative extraction in the 2.0 to 9.0 pH range. Protonation of the PDC anion, and subsequent degradation, are said to account for the reduced extraction efficiencies below pH 2.0.

The effect of initial aqueous Cd(II) concentration on the extraction efficiency is marked with a decrease in extraction efficiency beginning as low as 50 ppb cadmium. Factors investigated, and shown to contribute to the lowered

efficiency, include (1) insufficient APDC (as shown by APDC:Cd molar ratio data), and (2) saturation of the metal chelate in the organic phase. It is not clear which of these two processes is most important in depressing the extraction efficiency of Cd(II) in aqueous systems, but both contribute.

With these results in mind, one should be careful in extracting aqueous solutions containing >10 ppb cadmium using the standard APDC-MIBK procedure. The extraction of matched-matrix standards, along with unknowns, should help correct these problems.

ACKNOWLEDGMENTS

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Total Deposition of Various Elements from the Biscay Atmospheric Environment

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ABSTRACT

This paper contains data on the total and insoluble depositions of B, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn, Ti, V and Zn at industrial, urban and rural points of Biscay (Spain) over a period of one year. Samples were analyzed by spectrography after dilution with graphite powder and lithium carbonate. From the results it has been possible to establish those areas where the major depositions of certain elements generally occur, to estimate the sources of pollutant agents, to show their variations during the seasons of the year and to determine the solubility in water of constituents.

KEYWORDS

Seasonal Pollution, Dusts Fall Composition, Air Pollution, Total Deposition, Insoluble Deposition, Spectrographic Analysis, Inorganic Pollutants, Urban Air, Rural Air, Industrial Air.

INTRODUCTION

Because of the serious problems resulting from the ever-increasing environmental degradation, and since an important area of Biscay is significantly polluted, an extensive research programme has been carried out, for several years, at the Inorganic Chemistry and Chemical Analysis Department of the Escuela Superior de Ingenieros Industriales of Bilbao, in order to find out the contents of various chemical species in the atmospheric environment of Biscay. About ten thousand analytical data are presently available.

Bearing in mind that water is a good solvent for many atmospheric pollutants and that most of the determinations made on it do not present great difficulties, one of the goals of this research has been the evaluation of levels of atmospheric pollution by means of the analysis of rainwater, in those areas in which it rains frequently and pollution levels are important. With this objective, sampling devices were placed at points

representative of the industrial, urban and rural areas of Biscay. Seven day samples were collected and tested over a period of one year. Silica, Sulphates, nitrites, nitrates, ammonium, bicarbonates, chlorides, fluorides, Al, Li, Na, K, Ti, V, Ca, Mg, Cu, Zn, Mo, Ag, Sn, B, Cr, Mn, Fe, Co, Ni, Pb, Ga and Ge were determined by visible and UV spectrophotometry, conductrometric and potentiometric titrations, flame photometry, atomic absortion spectrophotometry and emission spectrography, after preconcentration of the samples, in most cases.

From the results included in several papers (Gracia and Elejalde, 1977a, 1977c, 1978; Gurtubay, Gracia and Elejalde, 1978a, 1978b, 1978c) it has been possible:

a) To establish a relationship between the concentrations of constituents of the rainwater, which has allowed the determination, in some cases, of the nature and source of the polluting substances, in order to explain why samples are most of times neutral, in spite of the fact that their sulphate contents, as well as sulphurous releases, are high in the involved area, etc.

b) To relate meteorological conditions (wind direction and speed, atmospheric pressure, relative humidity, temperature, periods of time without rainfall and size of drops with rain - water and environmental pollution).

c) To define the most polluted areas and to know the influence that pollution of the industrial areas has on both the rural region and the city of Bilbao. From the variation and dispersion of analytical results at several points, it has been possible to calculate the number of sampling devices necessary to esta - blish a future system of permanent inspection whithin the involved region.

Recent papers (Gracia and Elejalde, 1977b, 1977d) show the results of the concentration of Al, Li, Na, K, Ti, V, Cu, Zn, Mo, Ag, Sn, B, Mg, Cr, Mn, Fe, Co, Ni, Pb, Ga, Ge y Si in the dry deposition of the area. This data has confirmed and enlarged several conclusions obtained from rainwater. In this report, some data is provided on the constitution of the total deposi - tion of Biscay.

At present, the work essentialy leans towards establishing a relationship between concentrations of various pollutants in rainwater and in the atmospheric environment (atmospheric dusts and gases), which will permit not only to estimate, but also to determine levels of atmospheric pollution by means of the analysis of rainwater.

EXPERIMENTAL

Sampling

Six points were chosen, three in the industrial zone, two in the city of Bilbao and the last one in a rural area. Their location is shown in Fig. 1.

The main features are as follows:

Point 1 (Baracaldo). It is located in a typically industrial area, under the influence of iron and steel industry and nitrogen fertilizers manufacture and quite near a thermal power plant.

Point 2 (Basauri). It is situated in an industrial area where iron and steel works and chemical industries are numerous.

Point 3 (Ceberio). It corresponds to a typically rural place, surrounded by pieces of land devoted to timber farming.
Points 4 and 6. They are located at the centre of the city of Bilbao, on both sides of the river, point 4 corresponding to the side on which traffic is greater.
Point 5 (Erandio). It is placed in an industrial area essentially affected by the presence of non-ferreous metallurgical and chemical industries.

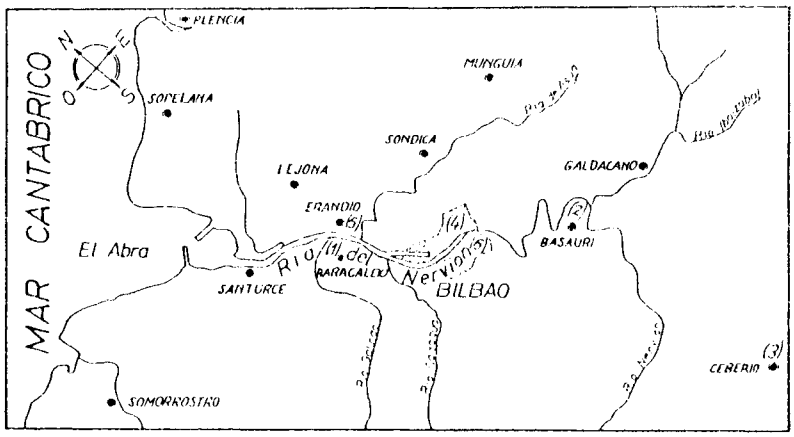

Fig. 1. Location of sampling stations

The devices used for the sampling consisted of a 250 mm diameter polyethylene funnel connected at its bottom to a 10 liters vessel of the same material
They were placed at the center of wide terraces, the height of which, with respect to the ground, was estimated between 10 and 15 meters.
The samples correspond to the total deposition, i.e. to the wet deposition plus the dry deposition, which, after settling on the funnel walls, was dissolved and driven down by the rainwater.
During one year, four week sampling periods were established.

Analytical Methods

For total deposition a previously homogenized aliquot was heated to dryness at 110º C in platinum dish, before being tested.
Another aliquot was filtered through a medium pore size glass filter crucible and the residue was also submitted to analysis after evaporation to dryness.
The samples were tested by spectrography after dilution with graphite and lithium carbonate in the relations 1:1.5:1.5.
Standard samples were prepared in the same way with "Spex Mix" (Spex Industries, Inc. Metuchen. N.J.).
The conditions of analysis were as follows:
Spectrograph: E 492 Automatic Hilger (quarz prism).
Source: Direct-current arc ARL, 120V, 10A.

Microphotometer: Hilger - Jaco, L-90.
Electrodes: Johnson-Matthey, spectral purity graphite. Lower electrode of 10 mm long with crater of 3,5 mm in diameter and 3 mm in depth.
Plates: Kodak SA-1 (10.2 x 25.4 cm).
Distance between electrodes: 2 mm.
Weight of sample: 20 mg.
Slit: 10 μ wide, 2 mm long.
Time of exposure: 45 seconds.
Spectral range: 2450 - 3500 Å.

RESULTS

Figures 2 and 3 represent the values of the annual total deposition of B, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn, Ti, V and Zn, expressed in $\mu g/cm^2$. yr, obtained from each one of the six sampling points. The figures shown with an arrow correspond to results higher than represented.

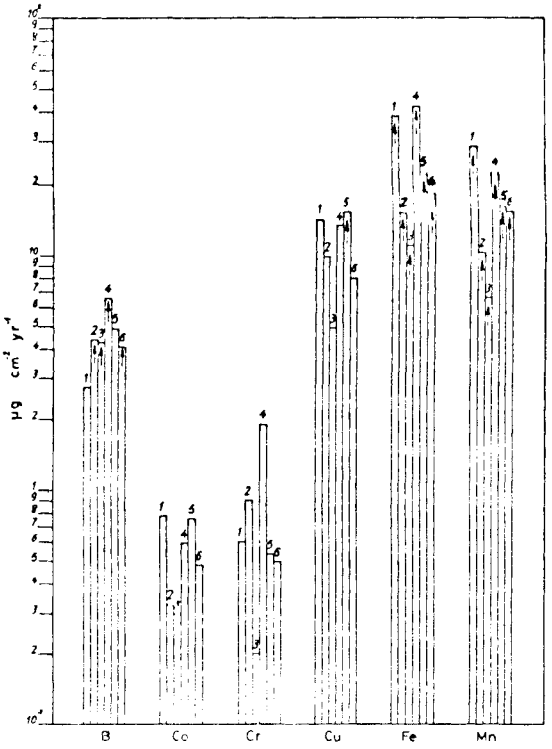

Fig. 2. Annual total deposition of B, Co, Cr, Cu, Fe and Mn at the sampling stations.

Table 1 shows the amounts of the above mentioned elements, deposited at point 1, in each of the four seasons of the year. This point has been chosen because it seems to be the most represen-

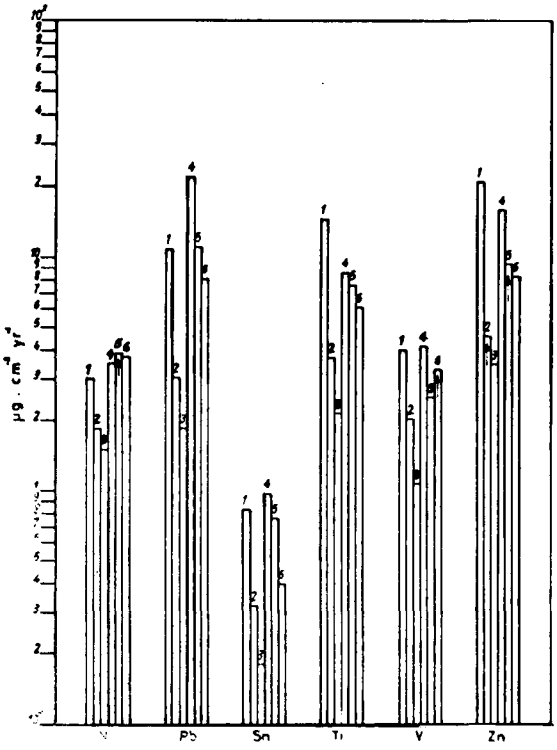

Fig. 3. Annual total deposition of Ni, Pb, Sn, Ti, V and Zn at the sampling stations.

TABLE 1 Total Deposition, mg/m^2, during the four Seasons of the Year (Point 1).

Elements	Winter	Spring	Summer	Autumn
B	7.0	4.0	8.5	6.8
Co	4.0	1.1	1.5	1.1
Cr	1.4	1.8	1.2	1.2
Cu	33	55	32	2.4
Fe	> 96	>93	>100	>72
Mn	>109	>80	> 67	>55
Ni	10	8	6	5
Pb	47	23	24	14
Sn	1.1	2.5	2.2	2.2
Ti	37	58	24	28
V	13.8	8.5	8.5	7.8
Zn	45	66	37	55

tative, because apart from the fact that it is situated in a particularly industrial area, it is also located within an important centre of population.

Figures 4 and 5 show the annual insoluble deposition of the elements considered in this report.

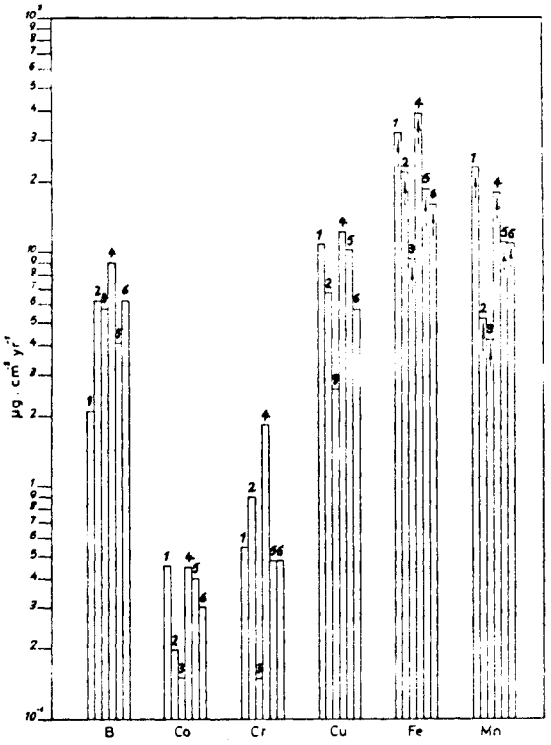

Fig. 4. Annual insoluble deposition of B, Co, Cr, Cu, Fe and Mn at the sampling stations.

DISCUSSION

The results obtained allowed us to make the following main considerations:

1) The major depositions of certain elements occur at the following points:

a) Copper and nickel at point 5, owing to the influence of non ferreous metal industry.

b) Lead and chromium at point 4, which is located on the busiest road of Bilbao and near an industry devoted to hard chromium coatings. At point 2, which is close to special steel manufacturing industries, very high chromium depositions occasionally appear.

c) Iron, manganese, titanium and zinc, at point 1, where most of the iron and steel industries of the region are situated. We would like to emphasize the fact that at point 4, an urban zone, considerable depositions of various elements generally occur. This is not surprising, since the predominant winds of the region come from the sea and, following the direction of the river, transport smoke and impurities from the area of great industrial activity towards Bilbao.

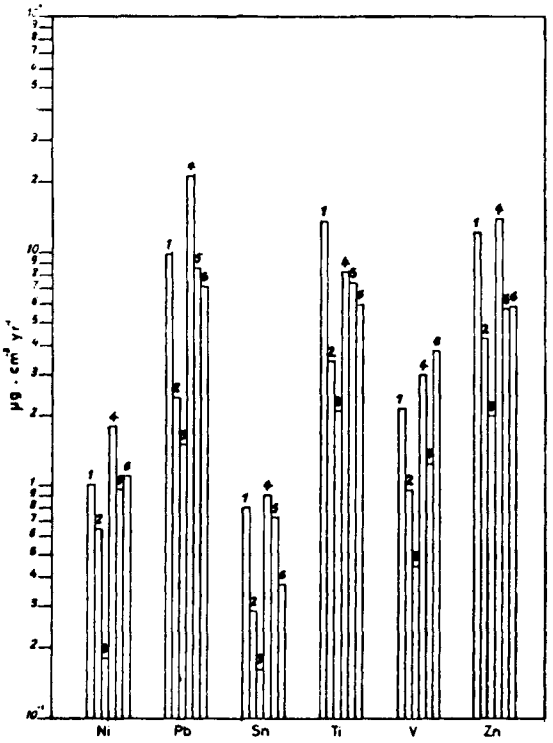

Fig. 5. Annual insoluble deposition of Ni, Pb, Sn, Ti, V and Zn at the sampling stations.

2) Despite the fact that the lowest depositions of the studied elements appear at point 3, we noticed that this is directly affected by pollution from both the industrial region and the urban zone.

3) The depositions of the considered elements, throughout the four seasons of the year, show generally, their highest values in Spring. Nevertheless, cobalt, nickel, lead and vanadium are frequently higher in winter. These results are in accordance with others authors who indicate that vanadium, lead and nickel are associated with combustion of coal and oil fuels, because of domestic heating. Kneip and others (1970) report an increase in V and Ni concentrations in New York during winter which they attribute to oil-burning sources. Elevated levels of V were found in Milan in winter by Rolla and others (1973). It should be pointed out that, in the concerned area, rainfall is more frequent and intense in Spring, so increasing the amount of pollutants dissolved and drawn by rainwater in this season.

4) Solubility in water of some elements of the total deposition is important with respect to their biological availability and their passage through the earths environment. The average solubility values obtained at the various sampling points, during the studied period, indicate that the most insoluble elements among those analyzed are Cr, Sn and Ti (solubility eqùal to or lower than 5 per cent). B and Pb presented values between 10

and 20%, Co and V of 40 - 45 %, Cu and Zn of 25 - 30 %, while solubility of Ni was estimated to be 65 %.
These results generally differ from those obtained in other countries. Investigations in urban Japan by Sadao and others (1970) established that percentages of soluble to total deposit were large for Cu, Mn and Zn (26-64%), small for Ni and Pb ($\sim$ 1%) and nearly zero for V, Cr and Co. On the other hand, Cawse (1974), taking 1972-73 averages at British stations, found 20 % solubility for Fe, 75 - 90 % for Zn and Pb, and 50 - 75 % for Cr, Mn, Co, Ni and Cu. This is not strange, considering that the solubility of these elements depends on the actual type of compounds.

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