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(520) 299-3735 (phone and FAX)

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Archives of Environmental Contamination and Toxicology

4213 Gann Store Road
Hixson, Tennessee 37343, USA
(615) 877-5418

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Foreword

International concern in scientific, industrial, and governmental communities over traces of xenobiotics in foods and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published research papers and progress reports, and archival documentations. These three international publications are integrated and scheduled to provide the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. This series is reserved exclusively for the diversified literature on "toxic" chemicals in our food, our feeds, our homes, recreational and working surroundings, our domestic animals, our wildlife and ourselves. Tremendous efforts worldwide have been mobilized to evaluate the nature, presence, magnitude, fate, and toxicology of the chemicals loosed upon the earth. Among the sequelae of this broad new emphasis is an undeniable need for an articulated set of authoritative publications, where one can find the latest important world literature produced by these emerging areas of science together with documentation of pertinent ancillary legislation.

Research directors and legislative or administrative advisers do not have the time to scan the escalating number of technical publications that may contain articles important to current responsibility. Rather, these individuals need the background provided by detailed reviews and the assurance that the latest information is made available to them, all with minimal literature searching. Similarly, the scientist assigned or attracted to a new problem is required to glean all literature pertinent to the task, to publish new developments or important new experimental details quickly, to inform others of findings that might alter their own efforts, and eventually to publish all his/her supporting data and conclusions for archival purposes.

In the fields of environmental contamination and toxicology, the sum of these concerns and responsibilities is decisively addressed by the uniform, encompassing, and timely publication format of the Springer-Verlag (Heidelberg and New York) triumvirate:

Reviews of Environmental Contamination and Toxicology [Vol. 1 through 97 (1962–1986) as Residue Reviews] for detailed review articles concerned with any aspects of chemical contaminants, including pesticides, in the total environment with toxicological considerations and consequences.

Bulletin of Environmental Contamination and Toxicology (Vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

Archives of Environmental Contamination and Toxicology (Vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

Manuscripts for *Reviews* and the *Archives* are in identical formats and are peer reviewed by scientists in the field for adequacy and value; manuscripts for the *Bulletin* are also reviewed, but are published by photo-offset from camera-ready copy to provide the latest results with minimum delay. The individual editors of these three publications comprise the joint Coordinating Board of Editors with referral within the Board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

Coordinating Board of Editors

Preface

Worldwide, anyone keeping abreast of current events is exposed daily to multiple reports of environmental insults: global warming (greenhouse effect) in relation to atmospheric CO_2, nuclear and toxic waste disposal, massive marine oil spills, acid rain resulting from atmospheric SO_2 and NO_x, contamination of the marine *commons*, deforestation, radioactive contamination of urban areas by nuclear power generators, and the effect of free chlorine and chlorofluorocarbons in reduction of the earth's ozone layer. These are only the most prevalent topics. In more localized settings we are reminded of exposure to electric and magnetic fields; indoor air quality; leaking underground fuel tanks; increasing air pollution in our major cities; radon seeping from the soil into homes; movement of nitrates, nitrites, pesticides, and industrial solvents into groundwater; and contamination of our food and feed with bacterial toxins. Some of the newer additions to the vocabulary include *xenobiotic transport*, *solute transport*, *Tiers 1 and 2*, *USEPA to cabinet status*, and *zero-discharge*.

It then comes as no surprise that ours is the first generation of mankind to have become afflicted with the pervasive and acute fear of chemicals, appropriately named *chemophobia*.

There is abundant evidence, however, that virtually all organic chemicals are degraded or dissipated in our not-so-fragile environment, despite efforts by environmental ethicists and the media to persuade us otherwise. But for most scientists involved in reduction of environmental contaminants, there is indeed room for improvement in all spheres.

Environmentalism has become a global political force, resulting in multinational consortia emerging to control pollution and in the maturation of the environmental ethic. Will the new politics of the next century be a consortium of technologists and environmentalists or a progressive confrontation? These matters are of genuine concern to governmental agencies and legislative bodies around the world, for many chemical incidents have resulted from accidents and improper use.

For those who make the decisions about how our planet is managed, there is an ongoing need for continual surveillance and intelligent controls, to avoid endangering the environment, wildlife, and the public health. Ensuring safety-in-use of the many chemicals involved in our highly industrialized culture is a dynamic challenge, for the old established materials are continually being displaced by newly developed molecules more acceptable to environmentalists, federal and state regulatory agencies, and public health officials.

Adequate safety-in-use evaluations of all chemicals persistent in our air, foodstuffs, and drinking water are not simple matters, and they incorporate the judgments of many individuals highly trained in a variety of complex biological, chemical, food technological, medical, pharmacological, and toxicological disciplines.

Reviews of Environmental Contamination and Toxicology continues to serve as an integrating factor both in focusing attention on those matters requiring further study and in collating for variously trained readers current knowledge in specific important areas involved with chemical contaminants in the total environment. Previous volumes of *Reviews* illustrate these objectives.

Because manuscripts are published in the order in which they are received in final form, it may seem that some important aspects of analytical chemistry, bioaccumulation, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology have been neglected at times. However, these apparent omissions are recognized, and pertinent manuscripts are in preparation. The field is so very large and the interests in it are so varied that the Editor and the Editorial Board earnestly solicit authors and suggestions of underrepresented topics to make this international book series yet more useful and worthwhile.

Reviews of Environmental Contamination and Toxicology attempts to provide concise, critical reviews of timely advances, philosophy, and significant areas of accomplished or needed endeavor in the total field of xenobiotics in any segment of the environment, as well as toxicological implications. These reviews can be either general or specific, but properly they may lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology. Certain affairs in food technology concerned specifically with pesticide and other food-additive problems are also appropriate subjects.

Justification for the preparation of any review for this book series is that it deals with some aspect of the many real problems arising from the presence of any foreign chemical in our surroundings. Thus, manuscripts may encompass case studies from any country. Added plant or animal pest-control chemicals or their metabolites that may persist into food and animal feeds are within this scope. Food additives (substances deliberately added to foods for flavor, odor, appearance, and preservation, as well as those inadvertently added during manufacture, packing, distribution, and storage) are also considered suitable review material. Additionally, chemical contamination in any manner of air, water, soil, or plant or animal life is within these objectives and their purview.

Normally, manuscripts are contributed by invitation, but suggested topics are welcome. Preliminary communication with the Editor is recommended before volunteered review manuscripts are submitted.

Department of Entomology
University of Arizona
Tucson, Arizona

G.W.W.

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Measurements of Environmental Lead Contamination and Human Exposure

A. Russell Flegal* and Donald R. Smith*,†

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I. Introduction

The enormous magnitude and extent of environmental and human lead contamination has become apparent over the last three decades, while the harmful effects of lead contamination on human and environmental health still may not yet be fully realized (Needleman 1992; NRC 1993; USEPA 1986). Lead contamination of the biosphere has occurred on a global scale

*Environmental Toxicology, WIGS, University of California, Santa Cruz, CA 95064, U.S.A.
†Department of Laboratory Medicine, University of California, San Francisco, CA 94110, U.S.A.

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for hundreds if not thousands of years, and despite efforts by many industrialized nations to reduce some lead emissions (e.g., lead alkyls in gasoline), lead production worldwide continues to increase (U.S. Bureau of Mines 1992). Much of our current state of knowledge on the magnitude of contaminant lead in the environment and on its toxicity at low levels of exposure has been derived from recent analyses using more sensitive analytical and clinical measurements of lead and its effects on organisms.

The global perturbation of the natural lead cycle went largely unnoticed until scientists, chiefly C.C. Patterson and his associates, demonstrated that order(s)-of-magnitude increases in environmental and human lead levels had occurred since preindustrial times (Boutron et al. 1991; Elias et al. 1982; Ericson et al. 1979; Flegal and Patterson 1983; Flegal and Smith 1992a; Murozumi et al. 1969; Patterson 1965; Patterson 1987; Patterson et al. 1987, 1991; Shaule and Patterson 1981; Shen and Boyle 1987; Shirahata et al. 1980; Smith et al. 1990, 1992a). All of these studies utilized what are now termed "trace metal clean" techniques during sample collection, processing, and analysis to avoid incidental contamination of the samples with lead. The studies demonstrated that many previously reported measurements of lead concentrations in the environment and humans were erroneously high by order(s) of magnitude due to the inadvertent contamination of samples (e.g., Everson and Patterson 1980; Flegal and Coale 1989; Manton and Cook 1984; Michaels and Flegal 1990; Settle and Patterson 1980).

As the global magnitude of lead contamination has been realized, it has raised new concerns about the chronic toxicity and sublethal effects of human lead exposures (NRC 1993; Smith and Flegal 1992b; USEPA 1986). These concerns are substantiated by studies that have shown that there may be no lower threshold concentration for lead toxicity in contemporary humans (Bellinger et al. 1987, 1991; Dietrich et al. 1992, 1993a,b; McMichael et al. 1988; Needleman et al. 1979, 1990; Schwartz 1994; Schwartz and Otto 1987; Wasserman et al. 1994). It should also be recognized that these concerns, while now well justified, were initially raised by C.C. Patterson three decades ago (Patterson 1965).

The lack of a currently discernible threshold for some measures of sublethal lead toxicity has been supported by the results of numerous studies documenting insidious effects of lead at concentrations that were previously considered innocuous (Bellinger et al. 1987, 1991; Dietrich et al. 1992, 1993a,b; McMichael et al. 1988; Schwartz and Otto 1987; Schwartz et al. 1986; Wasserman et al. 1994). Those studies have contributed to new (1991) U.S. Department of Health and Human Services Centers for Disease Control (CDC) guidelines in the United States that have dramatically lowered the level of concern for childhood lead poisoning to blood lead concentrations of ≥ 10 μg/dL, or 480 n$\underline{M}$ (CDC 1991). However, it was recently suggested (Flegal and Smith 1992a) that the natural blood lead concentration in humans (0.016 μg/dL, or 0.8 n$\underline{M}$) was 175-fold lower than the current (1991) average blood lead levels in the U.S. (2.8 μg/dL, or 140 n$\underline{M}$)

(Brody et al. 1994) and fully 600-fold lower than the recently (1991) revised CDC action level of concern for early toxic effects in children (10 μg/dL, or 480 nM).

The relative difference between the estimated natural blood lead concentration of humans and the latest CDC action level of concern for early toxic effects in children is disturbing (Smith and Flegal 1992b). While the blood lead levels that are now considered acceptable in children (i.e., <10 μg/dL, or <480 nM) are nearly 600-fold greater than the estimated natural level, they are only ≈ 10-fold lower than levels (≈ 100 μg/dL, or 4800 nM) that may cause encephalopathy and death in many individuals (Needleman 1992; USEPA 1986) (Fig. 1). These data suggest that adverse health effects of lead may extend to children with blood lead concentrations below the current CDC action level.

Although most of the early reports of lead concentrations in humans were limited to individuals suffering from acute lead toxicity, there have been studies within the past several decades that have focused on defining the sublethal effects of lead toxicity. A notable series of studies by Needleman and others have demonstrated neurobehavioral impairment in children

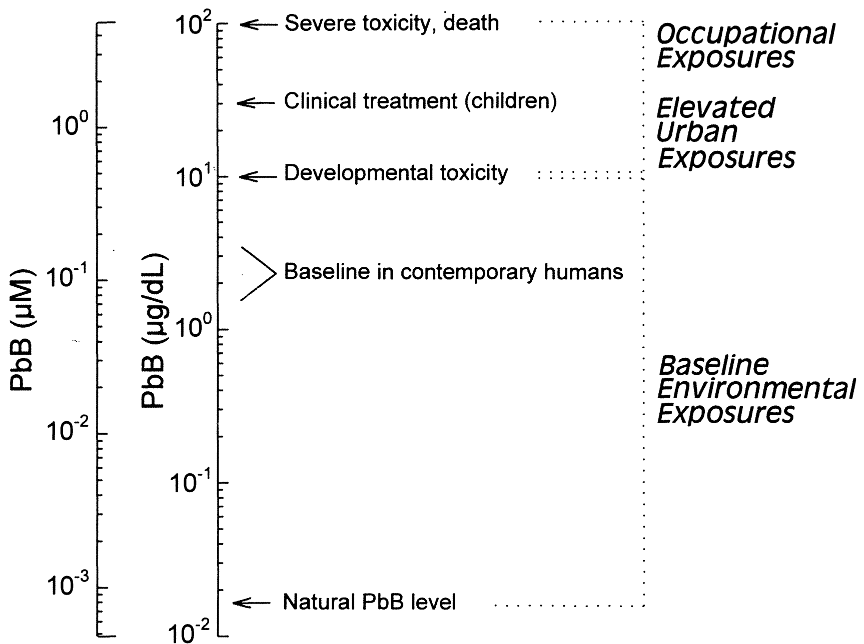

Fig. 1. Perspective on human lead exposures. Baseline blood lead levels in contemporary humans are >200-fold greater than natural levels, although only 2- to 4-fold lower than blood lead levels associated with developmental toxicity in children (>10 μg/dL) (Smith and Flegal 1992b, 1995).

with relatively low lead exposures (Bellinger et al. 1987, 1991; Dietrich et al. 1992, 1993a,b; McMichael et al. 1988; Needleman et al. 1979, 1990; Schwartz 1994; Schwartz and Otto 1987; Wasserman et al. 1994). Other studies have demonstrated subclinical effects of lead on hearing (Schwartz and Otto 1987), stature (Schwartz et al. 1986), and blood pressure (Cardoza dos Santos et al. 1994; Sharp et al. 1987). Combined, these studies demonstrate the insidious and pervasive nature of lead poisoning in the U.S. and other industrialized countries; they also suggest that the occurrence of lead poisoning in some developing countries may be comparable or higher, since environmental lead exposures are often greater in those countries with fewer regulations on lead uses and emissions.

Consequently, this review is designed to briefly summarize many of the available techniques for accurate measurements of environmental and human lead contamination. This includes the importance of ultraclean techniques for lead analysis as well as brief descriptions of some current and emerging analytical techniques for measuring lead exposures in humans. The descriptions are preceded by abbreviated discussions of the chemical properties of lead, natural and anthropogenic variations in its stable isotopic composition, and historical records of lead contamination in the environment. The report concludes with a summary of some indirect methods of measuring lead exposure and toxicity in humans. Much of the material in this report is based on reviews written for several recent reports: "Measuring Lead Exposure in Infants, Children, and Other Sensitive Populations" (NRC 1993), "Lead in the Biosphere: Recent Trends" (Smith and Flegal 1995), and "In Vivo Measurement and Speciation of Nephrotoxic Metals" (Smith and McNeill 1995).

II. Chemical, Biological, and Environmental Properties of Lead

Lead cations occur in both the divalent (+2) oxidation state, which is the most stable of the group IVB elements, and in the tetravalent (+4) oxidation state. The divalent oxidation state usually dominates the inorganic chemistry of lead, while the tetravalent state dominates its organic chemistry. The coordination numbers of its divalent compounds range from 2 to 7, while those of its tetrahedral compounds range from 4 to 8. Its stereochemistry is usually octahedral or tetrahedral.

Divalent lead (Pb^{2+}), although a "borderline" class metal, demonstrates primarily soft-sphere or B-type metal cation properties. The classification is based on the number of electrons in its outer shell and indicates that divalent lead cations tend to form covalent bonds. This contrasts with A-type or hard-sphere metal cations such as Ca^{2+}, which tend to form ionic bonds. As a borderline metal cation, Pb^{2+} has strong affinity for functional groups containing nitrogen and sulfur (e.g., CN^-, RS^-, SH^-, and imidazol), although it also forms important complexes with carboxyl group ligands in biological systems. Divalent lead is also present in geological de-

posits of galena (PbS), which is the most abundant lead ore, and in less common mineral deposits of anglesite ($PbSO_4$) and cerrusite ($PbCO_3$).

Since lead is particle active, it is efficiently scavenged by particles in many environmental and industrial lead cycling and emission processes (Church et al. 1990; Maring et al. 1987; Nriagu 1990a; Turekian 1979; USEPA 1986). This accounts for the effective transport of lead in particulate phases and its accumulation in microorganisms and sediments. It also accounts for the accumulation of lead near point sources of lead emissions, such as lead mines and lead smelters (Nriagu 1990b).

The cycling of lead through the biosphere results partly from its character as a calcium analog (Elias et al. 1982). Lead is somewhat similar in charge density (ionic radius and valence charge) to other alkaline earth elements, most notably calcium. As a biogeochemical analog to calcium, lead is readily incorporated into trophic and metabolic pathways (Burnett and Patterson 1980; Elias et al. 1982; Patterson 1982; Rosen and Pounds 1989). As a result, most (70% to >90%) of the lead in vertebrates is stored in mineralized tissues (e.g., the skeleton), where it is substituted for calcium in apatite matrices. Pb^{2+} cations may also compete for Ca^{2+} cation sites in other cellular and subcellular systems, including synaptosomes, mitochondria, membrane vesicles, protein kinase C, and calmodulin and other Ca^{2+}-binding proteins.

The presence of lead in many calcium-mediated cellular processes may account for many of the known mechanisms of lead toxicity. Several important mechanisms of toxicity are attributed to the alteration of calcium-mediated cellular processes and the alteration of regulatory proteins through the loss of functional and structural integrity (Pounds and Cory-Slechta 1993; Pounds et al. 1991). Lead also replaces other divalent cations in biological systems, including Zn^{2+} in δ-aminolaevulinic acid dehydratase, leading to the inhibition of that enzyme. However, there are sufficient differences in sizes and mass/charge ratios between these ions to preclude the Pb^{2+}-Zn^{2+}-Ca^{2+} interaction hypothesis as a fundamental basis for the specific effects of lead in biological systems.

Because the natural physiological distribution of lead across trophic levels generally follows that of calcium, lead concentrations across trophic levels may be normalized to calcium concentrations to facilitate evaluating intertrophic level perturbations with lead (Elias et al. 1982; Patterson 1965). This has been demonstrated for lead in discrete tissues within an organism (Burnett and Patterson 1980; Smith et al. 1992b), different organisms within a population (Ericson et al. 1979, 1990; Patterson et al. 1987, 1991), and different trophic levels in food chains (Elias et al. 1982; Michaels and Flegal 1990; Smith et al. 1990, 1992a). Normalizations of lead to calcium, which most appropriately use the atomic ratios of those elements, show a systematic decrease of lead concentration relative to calcium with ascending trophic level transfers as well as in calcium-rich tissues (e.g., the skeleton) of organisms relative to other tissues (blood, kidney). It is apparent from

those normalizations that lead is biologically depleted relative to calcium during the metabolic transfer of these elements. This biodepletion is attributed to a biological discrimination against lead in favor of calcium in the physiological processes that control calcium uptake and homeostasis (Burnett and Patterson 1980; Smith et al. 1990, 1992a,b).

However, the natural biodepletion process, which normally reduces the ratio of lead to calcium with ascending trophic transfer, has been circumvented by direct exposures of organisms at all food web levels to contaminant industrial lead in the environment. For humans, this occurs via numerous routes (Flegal et al. 1990; NRC 1993; USEPA 1986). Humans inhale lead aerosols derived from the combustion of gasolines with tetraethyl lead additives, fossil fuel combustion, and leaded dusts; ingest lead-containing soil, dust, and water contaminated by lead from numerous sources, including mines, smelters, leaded gasolines, lead-based paints, lead pipes, lead crystal, and lead solder; and consume foods contaminated by lead-soldered cans, lead utensils, and lead glazes. Because these additional exposures may swamp natural lead biodepletion mechanisms, the ratios of lead to calcium in human tissues may exceed those in their diet.

III. Isotopic Compositions of Lead

Lead exists naturally as four stable isotopes: ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb. Measurable differences in the relative abundances of these four isotopes throughout the environment result from the amounts and radioactive decay of the progenitor isotopes ^{238}U ($t_{1/2} = 4.5 \times 9^{10}$ yr), ^{235}U ($t_{1/2} = 0.70 \times 10^{9}$ yr), and ^{232}Th ($t_{1/2} = 1.4 \times 10^{10}$ yr) that form ^{206}Pb, ^{207}Pb, and ^{208}Pb, respectively (Faure 1986). The fourth stable lead isotope, ^{204}Pb, has no long-lived radioactive parent. Therefore, natural radioactive decay leads to variations in stable lead isotopic compositions in geological formations with different ages, parent–daughter isotope ratios, and weathering processes (Doe 1970).

Natural differences in lead isotopic compositions in geological formations persist after the lead has been extracted and processed as industrial lead. This occurs because there is no measurable biological, chemical, or physical fractionation of lead isotopes either in the environment or in industrial processes (Barnes et al. 1978; Flegal and Stukas 1987; Russell and Farquhar 1960). Although stable lead isotopic compositions may vary naturally by as much as 10%–15%, they generally vary less than that.

There are, however, relatively pronounced regional and temporal differences in the lead isotopic compositions of contaminant leads in the environment. These have often reflected differences in the isotopic compositions of lead alkyl additives in gasolines, as well as differences in other industrial emissions of lead (Elias et al. 1982; Flegal et al. 1989; Patterson and Settle 1987; Manton 1985; Rabinowitz 1987; Rabinowitz and Wetherill 1972; Smith et al. 1990, 1992a; Sturges and Barrie 1987; Tera et al. 1985). These

differences in isotopic compositions have allowed stable lead isotopes to be used as tracers of environmental and human lead metabolism.

IV. Environmental Lead Contamination

The magnitude of lead contamination in the environment is high relative to that of any other trace element (Nriagu and Pacnya 1988). On a global scale, most trace element cycles have not been substantially affected by anthropogenic processes, but those processes have greatly perturbed the natural lead cycle (Nriagu and Pacnya 1988). This is due to the extensive processing of lead ores, which has released ≈ 300 million metric tons of contaminant lead into the environment during the past five millennia (Fig. 2). For example, global atmospheric emissions of industrial lead aerosols are estimated to be $\approx 3.3 \times 10^8$ kg/yr (Nriagu and Pacnya 1988). Those anthropogenic emissions are 700-fold greater than estimated aerosol emissions from natural processes (Nriagu, 1989; Patterson and Settle 1987).

Prior to the early 1990s, much of the lead released into the biosphere was deposited around mining and smelter operations, which were the primary sources of industrial lead emissions (Nriagu 1990b). That dispersion pattern changed with the advent of lead alkyl gasoline additives in the U.S. in the early 1920s and their subsequent worldwide proliferation. Combustion of leaded gasolines, which often contained more than 0.5 g Pb/L before regulation, became the single most significant source of global lead emissions to the atmosphere within the U.S. and many other countries (Fig. 3). It has

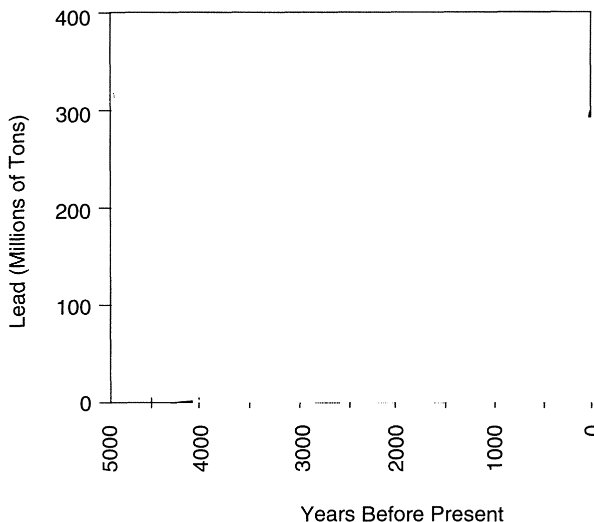

Fig. 2. Cumulative production and discharge of lead to the environment over time (from Flegal and Smith 1992b).

been estimated that 4–5 million metric tons of lead have been deposited in the environment in the U.S. since the introduction of alkyl lead additives in the early 1920s (USEPA 1986).

Although the majority of fallout from atmospheric lead emissions has occurred within kilometers of the region of discharge, some lead aerosols were entrained into upper atmosphere winds and transported large distances across international boundaries (Boutron et al. 1991; Church et al. 1990; Flegal et al. 1989; Maring et al. 1987; Smith et al. 1990; Veron et al. 1994). This was first evidenced in Greenland ice cores (Fig. 4), which exhibited about a 200-fold increase in lead concentrations since ancient times due to increases in global lead emissions during the past 5000 years (Boutron et al. 1991). Increases of comparable orders of magnitude have occurred in remote alpine pond sediments (Shirahata et al. 1980), marine sediments (Ng and Patterson 1982), and ocean corals (Shen and Boyle 1987). Similarly, contemporary baseline levels of lead in many organisms exceed natural levels by orders of magnitude. Therefore, it is estimated that more than 95% of the lead now in the biosphere is derived from industrial sources.

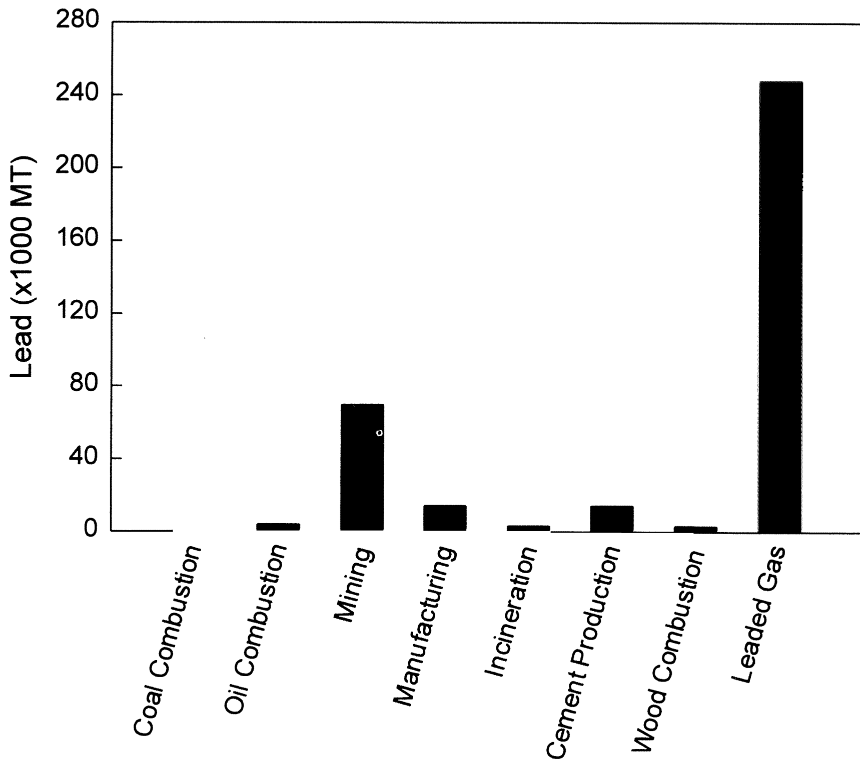

Fig. 3. Worldwide lead emissions to the atmosphere by category of use in 1983 (Nriagu and Pacnya 1988; from Smith and Flegal 1995).

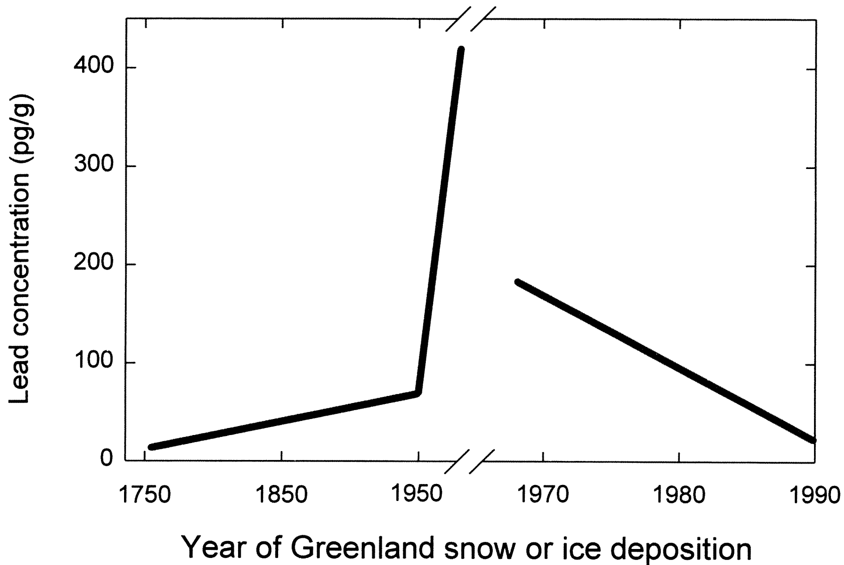

Fig. 4. Temporal changes in lead concentrations in Greenland snow and ice (Boutron et al. 1991; from Smith and Flegal 1995).

The extensive use of lead in industry and consumer materials, and the predominance of regional (versus global) dispersion patterns, has resulted in severe contamination of urban areas (Fig. 5). Much of that lead has accumulated in dusts and soils, which have proved to be an important source of exposure for urban residents, particularly children. Worldwide, a large number of consumer products (e.g., batteries, gasolines, paints, solders, pipes, ceramics, dyes, glazes, and construction materials) still contain high levels of lead, and lead released from those products continues to contaminate the environment (USEPA 1986). Most of that lead cannot be economically recovered using current technology after it has been released to the environment (Nriagu 1990a). Consequently, humans continue to be exposed to levels of lead that greatly exceed natural exposures (NRC 1993; Patterson 1965; USEPA 1986).

However, recent regulation of several lead-containing products (e.g., leaded gasoline, paint, and solder) in the U.S. and elsewhere has reduced the consumption of lead in some materials. For example, lead alkyl additives for gasolines, which accounted for approximately 20% of the lead consumed in the U.S. during the 1970s, are no longer used there. Although many other countries have also adopted regulations to reduce or eliminate the lead in gasoline (Nriagu 1990a), the use of lead alkyl additives is still increasing in many developing countries.

Reductions in the amount of lead in gasolines and other industrial sources have had a positive effect on lowering some lead emissions to the

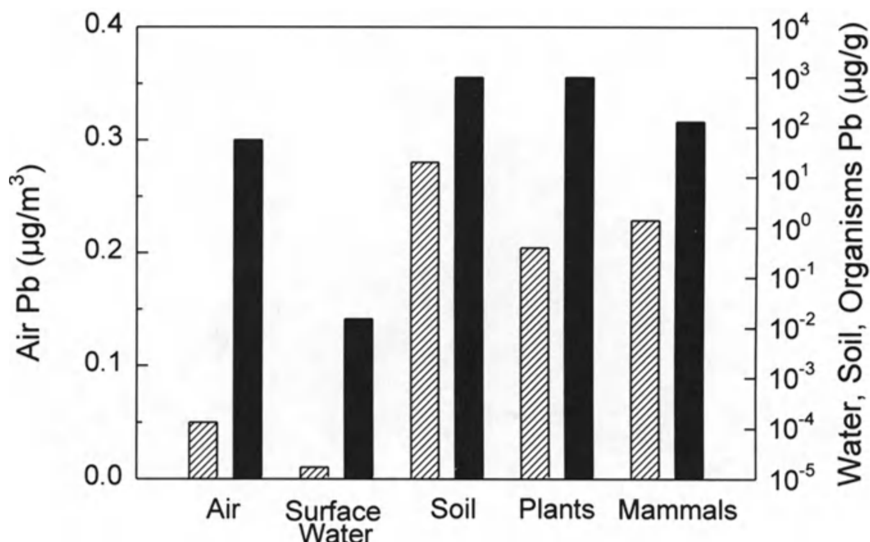

Fig. 5. Environmental and organismal lead concentrations in remote areas (*shaded bars*) versus urban areas (*solid bars*) (Flegal et al. 1990; from Smith and Flegal 1995).

environment (Boutron et al. 1991; Smith and Flegal 1995). This is evident in urban and suburban aerosol lead concentrations, which have declined markedly in recent years (USEPA 1986). It is also evident in the Greenland snow pack discussed above, which has shown a 7.5-fold decrease in lead concentrations during the past 20 years (Fig. 4). As a result, human lead exposures from environmental sources have also declined markedly during the past 15 years, particularly in the U.S. (Brody et al. 1994; Smith and Flegal 1995). These decreases demonstrate that regulatory control of lead use can have a direct effect on reducing additional lead inputs to the biosphere and human exposures.

Despite those reductions, the total U.S. and worldwide consumption of lead is not declining (Fig. 6). Lead consumption in products related to the transportation industry continues to increase, particularly in the production of lead acid batteries, which now account for nearly 80% of the lead consumed in the U.S. (Figs. 6 and 7). This indicates that new and proposed limits on some lead uses (e.g., leaded gasoline) will not be sufficient to alleviate the environmental lead contamination problem.

V. Measurement Methodologies

A. Background

As noted previously, this brief summary of methodologies is based primarily on two recent reviews (NRC 1993; Smith and McNeill 1995). Since the first of those reviews was completed, the utility of several of the experimen-

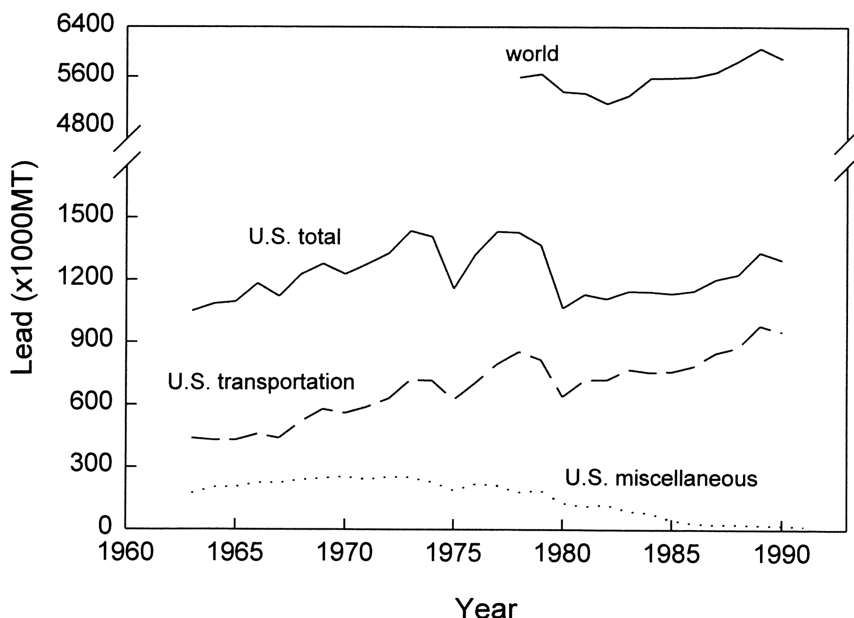

Fig. 6. Lead consumption in the United States and worldwide (U.S. Bureau of Mines 1992; from Smith and Flegal 1995).

tal methodologies described therein has been demonstrated. Notable among these are applications of microgram doses of stable lead isotopic tracers in laboratory and clinical studies to investigate the efficacy of therapeutic treatments of environmental lead contamination (e.g., Smith and Flegal 1992a; Smith et al. 1994), as well as advances in coupled methodologies, such as high performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICPMS), for determination of lead biomolecular speciation (Brown et al. 1994; Gercken and Barnes 1991; Owen et al. 1992; Smith and McNeill 1995). With the continuing development of new methodolgies, we assume that additional applications of existing or recently developed methods will occur by the time this review is published.

As noted throughout this review, investigators have encountered substantial problems with the inadvertent introduction of contaminant lead. This contamination occurs during collection, storage, processing and analysis. The specific procedures, now termed "trace-metal-clean techniques," that are required to circumvent those problems were detailed three decades ago by Patterson (1965), two decades ago by Patterson and Settle (1976), and more recently by Flegal and Smith (1992b).

The importance of these procedures in generating accurate data cannot be overstated, as is evidenced by analyses of the apparent 1000-fold decreases of lead concentrations in seawater (Bruland 1983) and freshwater

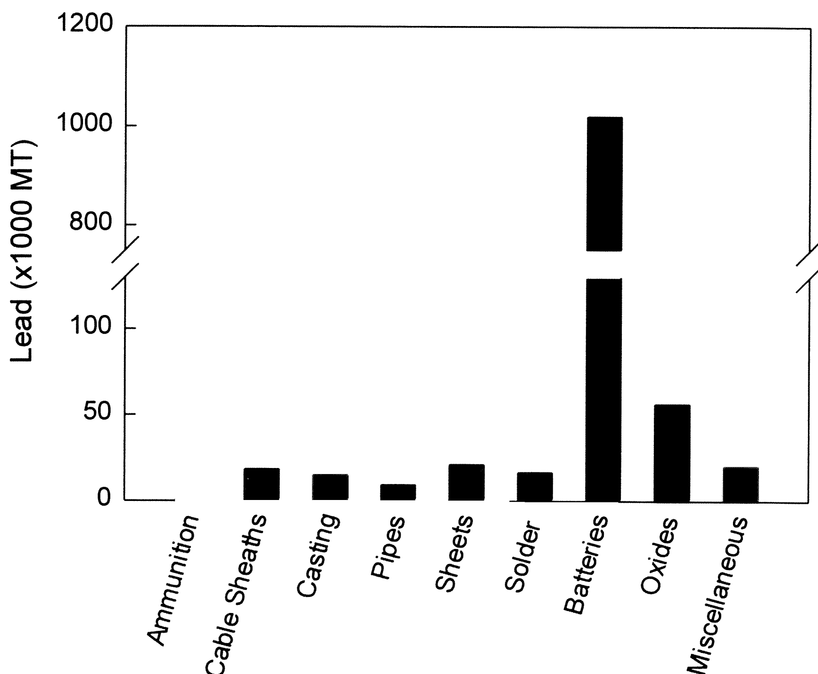

Fig. 7. Lead use by category in the United States in 1991 (U.S. Bureau of Mines 1992; from Smith and Flegal 1995).

(Flegal and Coale 1989; Windom et al. 1991) during the past four decades. In both cases, this has been identified as an artifact of successive reductions in sample contamination rather than a consequence of decreased inputs of industrial lead.

Trace-metal-clean techniques are also necessary in analysis of clinical samples with relatively low lead concentrations. This is illustrated by the relative contribution of contaminant lead in measurements of elevated (50 μg/dL) and low (1 μg/dL) PbB (Fig. 8). Moreover, the importance of these techniques will increase in clinical settings with projected declines in environmental lead exposures to humans in the U.S. and elsewhere (Brody et al. 1994; Flegal and Smith 1992b; Smith and Flegal 1995).

B. Problems with Accuracy and Precision

While the stringent procedures required to control lead contamination of samples have been detailed in several reports (e.g., Flegal and Smith 1992b; NRC 1980, 1993; Settle and Patterson 1980; Versieck et al. 1982) in addition to those previously cited, many laboratories still have not adopted these requisite procedures for measuring low levels of lead. Analytical reagents and inadequately cleaned labware, as well as contaminated sample

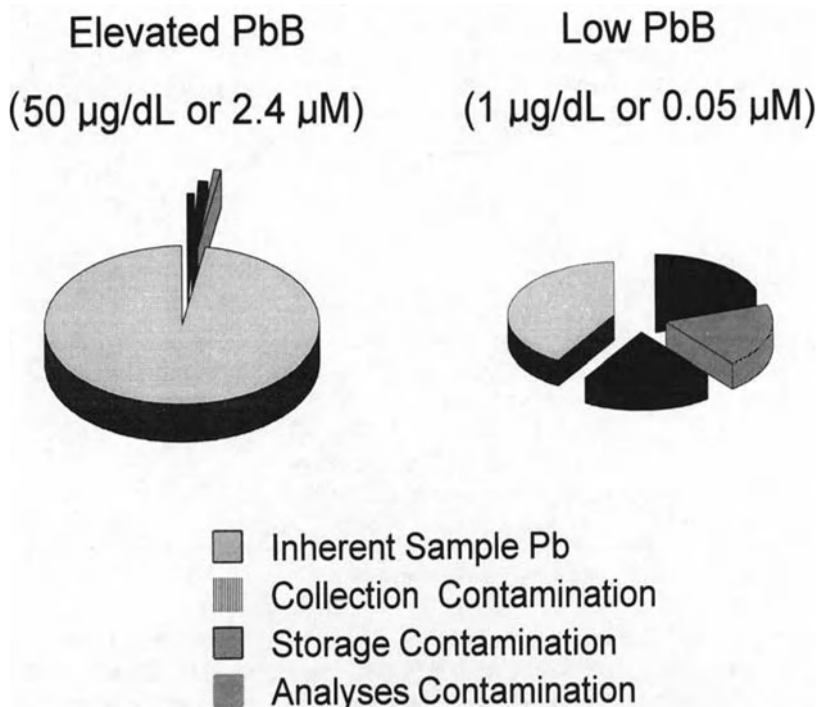

Fig. 8. Illustration of lead contamination bias introduced during collection (25 ng or 0.12 nmols Pb), storage (25 ng or 0.12 nmols Pb), and analysis (25 ng or 0.12 nmols Pb) of elevated (50 μg/dL or 2.4 μM) and low (1 μg/dL or 0.05 μM) blood lead samples (5 mL). That contamination (75 ng or 0.36 nmols Pb) increases the lead concentration of the elevated-lead sample by only 3%, but it increases the lead concentration of the low-lead sample by 150% (from Flegal and Smith 1992b).

collection containers (e.g., blood collection tubes), continue to be significant sources of contamination for low lead samples (Crick and Flegal 1991; Everson and Patterson 1980; Flegal and Smith 1992b; Hall et al. 1988; Moyer et al. 1991), as illustrated in Fig. 8. This is unnecessary, since most reagents can either be purchased in a "trace metal analyses" grade or be purified using routine procedures (e.g., cation/anion resin exchange or liquid-liquid extraction) (Table 1). Properly cleaned labware made of quartz, low-density polyethylene (LDPE), or tetrafluroethylene (Teflon®, PFA) has been shown to contribute negligible amounts of contaminant lead to the sample (Table 1). Additionally, trace-metal-clean facilities for conducting the analyses may be readily constructed with commercially available materials, including plastic laminar flow exhausting acid hoods and high-efficiency particle attenuation (HEPA) air filters.

Spurious contamination of samples with extraneous lead is in many cases a significant source of imprecision in measurements of low blood lead levels (<5 μg/dL). This source of imprecision is not unique to measurements of

Table 1. Lead contamination blanks for serum collection, processing, and analysis by isotope dilution thermal ionization mass spectrometry in the WIGS Trace Metal/Mass Spectrometry facility, University of California, Santa Cruz. Lead values are based upon repeated measurements.

	Pb source	Pb (pg)
Serum collection[a]	Butterfly catheter	5
	Polyethylene collection tube	2
Serum processing	5 mL HNO_3	12
	4.5 mL HBr	13
	2 mL HCl	6
	0.05 mL $HClO_4$	<1
	1 mL MQ H_2O	3
	AG-1 X8 resin	3
	Teflon columns and containers	4
Serum analysis	Mass spectrometer loading (Si gel, H_3PO_4)	5
Total Pb blank		53

[a]Assumes no Pb contribution from lysed erythrocytes.

lead in blood, but rather is a common problem in trace constituent analyses (Horwitz et al. 1980; Versieck et al. 1987). Imprecisions in many analyses (e.g., CV $\geq$ 20% for blood lead measurements) are contributed by the precision of measurements inherent to the analytical methodology. While these levels of precision may be adequate for identifying persons with elevated exposures such as encountered in public health settings, they may be inadequate for distinguishing blood lead levels among environmentally exposed individuals. This includes cases in the U.S., where baseline blood lead levels have declined to 2.8 μg/dL (Brody et al. 1994).

C. Isolation and Concentration

Lead measurements are often preceded by isolation and concentration procedures (Iyengar 1989). The former procedures reduce or eliminate analytical interferences, and the latter increase levels of analyte to measurable concentrations. Analyses of lead in water and biological matrices typically require the destruction of organic matrices with strong acids (e.g., HNO_3, aqua regia) and oxidants (e.g., H_2O_2 and $HClO_4$). In addition, analyses of total lead concentrations in geological matrices require the dissolution of silicate matrices with hydrofluoric acid. Measurements of lead concentrations in operationally defined fractions of biological and geological matrices are often derived from selective extractions (e.g., 0.5 N HCl, $HC_2H_3O_2$).

These initial treatments are followed by procedures to further isolate and concentrate the lead. Liquid–liquid extractions are most common for analyses of lead concentrations in aqueous matrices. These extractions typically use a water-immiscible solvent, an aqueous solution, and a complexing agent that forms a nonionic or neutral chelate with lead. A second type of

common extraction procedure uses ion-exchange resins, which sequester lead ions on chelating functional groups immobilized on a solid substrate. A third type of extraction is electrodeposition, which utilizes an electrode either to concentrate lead for direct electrochemical analyses or to preconcentrate it for another type of instrumental analysis.

D. Colorimetric Methods

Historically, lead concentrations were routinely measured with colorimetric methods using spectrophotometry. Currently, the primary spectrophotometric method for measuring lead is with dithizone (diphenylthiocarbazone). It is a relatively simple and inexpensive technique, but it is relatively insensitive. The accuracy and precision of this, as well as other colorimetric measurements of lead, are also highly dependent upon the skills of the analyst. Moreover, colorimetric measurements of lead in biological materials require the same level of ultraclean techniques that are necessary for more sensitive analytical methods.

E. Spectrometric Methods

The most common methods for measuring lead in biological and other environmental matrices are those based upon atomic absorption spectrometry (AAS) and (CPMS) (discussed in Section V. J). Atomic absorption spectrometry measures lead concentrations from the absorbance of lead spectra emitted from a source lamp by lead atoms vaporized into the light path of the source lamp (Slavin 1988). Flame AAS techniques are appropriate for measurements of relatively high (μg/mL) analyte concentrations of lead. Flameless AAS techniques are required for low (e.g., low ng/mL or less) level measurements. These include graphite furnace (GFAAS) measurements of low lead concentrations in complex matrices, such as blood, and they may be enhanced with methodologies using a L'vov platform and Zeeman-effect background correction. In our laboratory, the detection limit for GFAAS measurements of lead extracted from blood is typically 0.05 ng/mL.

Another common spectrometric method for measuring lead is atomic emission spectrometry (AES). It is similar to AAS, except that the lead is measured by the spectra of excited ions in the analyte. Atomic emission spectrometry measurements of lead are much less sensitive than GFAAS measurements. For example, we routinely reanalyze ICP-AES measurements of lead concentrations with another method (GFAAS or TIMS) when the concentration is less than 0.1–1 μg/mL because our experience has demonstrated that ICP-AES measurements of lead in that range are relatively inaccurate.

F. Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) techniques that have been developed for diagnostic analyses of lead in biological matrices and organisms

(Wrackmeyer and Horchler 1989) include the *in vivo* measurement of lead and lead complexation in tissues and cell cultures. A principal benefit of *in vivo* NMR spectroscopy is its usefulness in determining the biomolecular speciation of lead, as well as other metals, in a preselected region of tissue (or cells) if the metal species is NMR active. Measurements in tissues have demonstrated the applicability of *in vivo* NMR for the study of lead and other metals (e.g., Cd and Hg), including the characteristics of ligand interactions and mechanisms of toxicity in tissues (Arkowitz et al. 1987; Gartland et al. 1989; Jones et al. 1988; Long et al. 1994; Pesek and Schneider 1988; Reid and Podanyi 1988; Schanne et al. 1989a,b; Spencer et al. 1985; Vasak et al. 1985). These studies have substantiated the concept that particular biomolecular species of metals elicit specific effects that may manifest into overt cellular or organ toxicity.

Nuclear magnetic resonance has been used by Rosen and colleagues to investigate the toxic effects of lead in cell preparations. Their studies used ^{19}F-NMR and the divalent cation indicator 1,2-*bis*(2-amino-5-flurophenosy)-ethane-*N*,*N*,*N'*,*N'*-tetra acetic acid to investigate the levels of biologically labile lead within cultured cells, as well as the effects of lead on other calcium-mediated cellular processes (Dowd and Gupta 1991; Long et al. 1994; Schanne et al. 1989a,b). Other studies on the binding characteristics of lead and mercury to hen egg-white lysozyme demonstrated site-specific binding of lead and mercury, which proved to be different than the binding of methyl mercury (Pesek and Schneider 1988). Collectively, these studies evidence the applicability of *in vivo* NMR to investigate free and complexed lead levels within the cell as well as the effects of lead on normal cellular processes.

G. Electrochemical Methods

There have been several advances in electrochemical techniques to measure lead concentrations and its organic complexation in aqueous systems. The techniques, which have proven particularly useful for measuring lead concentrations in blood, are based on quantitation of the current produced as lead is reduced or oxidized by varying the potential of a working electrode in an electrochemical cell containing the sample. Electrochemical techniques can also be used to investigate the organic complexation of lead in dilute aqueous solutions, which is determined by titrating the sample with lead and measuring the current produced after each lead addition (e.g., Bruland et al. 1985).

The two principal electrochemical methods for measuring lead concentrations in environmental matrices are differential pulse polarography (DPP) and anodic stripping voltammetry (ASV). Differential pulse polarography measures the faradaic current produced by the reduction of Pb^{2+} to Pb^0 while the potential on the working electrode is scanned to negative potentials (Angerer and Schaller 1988). Anodic stripping voltammetry meas-

ures the lead after it has been preconcentrated on a mercury electrode by the reduction of Pb^{2+} to Pb^0 during a reducing step, and the oxidation current is then measured as the potential is then scanned over a positive range. Anodic stripping voltammetry is the preferred method for blood lead analyses because a relatively high level of sensitivity may be obtained (Feldman et al. 1994; Jagner 1982; Osteryoung 1988).

A notable recent advancement in ASV measurements of low lead levels (<10 μg/dL) in human blood samples is the use of disposable carbon microarray electrodes (Feldman et al. 1995). This methodology requires minimal sample pretreatment and provides a 10-fold improvement in the signal-to-noise ratio over single carbon disk electrodes (10-μm diameter), resulting in a detection limit of 5 μg/L for a 60-sec deposition. This new methodology may be adapted for public health programs that are responsible for screening large numbers of children for elevated (≥ 10 μg/dL or 480 nM) lead exposure, since it is relatively simple, inexpensive, and portable. It may also serve as an alternative to other currently used measurement techniques that are less sensitive (e.g., single carbon disk electrode ASV) or are relatively expensive and nonportable (e.g., GFAAS and ICPMS).

H. Chromatographic Methods

A wide range of lead molecular species may be measured when detector methodologies are coupled with chromatographic separation techniques. These techniques isolate the species of interest from the sample in a thermally and physicochemically stable form for separation by gas- or high-performance liquid chromatography (HPLC). Ion chromatography systems may then be coupled with thermal conductivity detectors (TCD), flame ionization detectors (FID), electron-capture detectors (ECD), thermionic-specific detectors (TSD), atomic emission spectrometers (AES), flame or graphite furnace AAS, or ICPMS.

There have been several adaptations of those methodologies to investigate the speciation of lead in the environment. Gas chromatography has been used to detect organolead compounds, including trialkylead chlorides. Gas-liquid chromatography was initially used to measure tetraalkylead derivatives in the environment, and it has subsequently been used to investigate the biomethylation of inorganic and ionic alkylead by microorganisms. Additional measurements of lead speciation in environmental and biological matrices may be obtained by coupling HPLC with GFAAS or ICPMS. Some of those coupled methodologies (e.g., HPLC–ICPMS) are discussed in the section on hybrid techniques.

I. X-ray Fluorescence

The development over the past several decades of x-ray fluorescence (XRF) instrumentation that is capable of measuring bone lead levels noninvasively has contributed to evaluations of body lead burdens and long-term lead

exposures in humans (Ahlgren et al. 1976; Christoffersson et al. 1984; Rosen et al. 1989; Sommervaille et al. 1988; Todd and Chettle 1994). The utility of *in vivo* XRF for assessing bone lead stores has been well demonstrated in lead-exposed populations (e.g., Ahlgren et al. 1976; Christoffersson et al. 1984; Rosen et al. 1989; Sommervaille et al. 1988; Watanabe et al. 1994). The *in vivo* measurement of soft tissue (e.g., kidney) lead levels is also being investigated (El-Sharkawi et al. 1986; Todd et al. 1993). Although the *in vivo* measurements of lead in bone will not replace any existing measurement of lead exposure in humans, they will supplement the existing tests by providing information on lead pools that cannot be otherwise sampled except by biopsy (Todd and Chettle 1994). There still are a number of concerns regarding the sensitivity, accuracy, and precision of XRF for assessing bone (or soft tissue) lead stores in environmentally exposed persons (NRC 1993; Smith and McNeill in press; Todd and Chettle 1994).

XRF instruments can be divided into two general classes, those that measure K-shell x-rays (K-XRF) and those that measure L-shell x-rays (L-XRF) (Todd and Chettle 1994). Although some consider the utility and efficacy of both methods to be similar, the K-shell instruments measure lead to a greater depth within the tissue than do the L-shell instruments. Because this is primarily the result of differences in the attenuation of the characteristic x-rays emerging from the tissue, L-XRF and K-XRF measurements sample different lead pools within the same measurement region. Therefore, complementary measurements with both types of instruments may be utilized to distinguish lead distributions within skeletal regions.

J. Mass Spectrometry

Mass spectrometry is a powerful class of analytical instrumentation capable of separating and detecting single and molecular charged ions. Significant advances in the performance characteristics of mass spectrometry began in the 1930s in studies of the focusing properties of electric and magnetic fields (Herzog and Mattuach 1934) and later in comparable improvements in vacuum technology, culminating in the availability of commercial instruments in the 1950s (Duckworth et al. 1986). Since that time, numerous instrument technologies well suited for measuring lead levels and stable lead isotopic abundances in environmental and biological matrices have been developed such as thermal ionization magnetic sector mass spectrometry (TIMS), ICPMS (both quadrapole and magnetic sector), glow discharge mass spectrometry (GDMS), and laser microprobe mass analysis spectrometry (LAMMA). The following sections briefly summarize some aspects of current and projected applications of mass spectrometers for analyses of lead concentrations and isotopic composition in environmental toxicology.

Thermal Ionization Mass Spectrometry (TIMS). TIMS continues to be the standard method by which all other lead concentration measurements are evaluated (NRC 1993). TIMS is considered the definitive method for accuracy in elemental analyses using isotope dilution mass spectrometry (IDMS), and it provides unprecedented precision, sensitivity, and detection limits for lead concentration analyses (Heumann 1988). It also has been the method used to demonstrate that many previously reported lead concentrations in environmental and biological samples were orders of magnitude higher than the true values due to sample contamination and analytical inaccuracies (e.g., Ericson et al. 1979; Everson and Patterson 1980; Flegal and Coale 1989; Patterson 1965; Settle and Patterson 1980).

Lead concentration measurements by IDMS are complemented by stable lead isotopic abundance measurements, which are also made by mass spectrometry. Lead isotope abundance measurements have found particular utility in environmental toxicology because isotopic compositions may be used to identify different sources (natural and industrial) of lead, which can often be distinguished by their isotopic composition (Faure 1986). In addition, stable lead isotopic tracers may be used to investigate lead cycling through food webs (Smith et al. 1990, 1992a) as well as lead metabolism within organisms (Rabinowitz et al. 1973, 1976, 1977; Smith and Flegal 1992a; Smith et al. 1992b, 1994).

The primary advantages of stable isotope tracer methodologies are their inherent sensitivity (picogram amounts of sample lead), their high level of precision (isotope ratio measurement errors of $\pm \leq 0.05\%$ RSE), and their ability to distinguish sample lead that originated from different sources (Smith et al. 1992b, 1994, in manuscript). Because very little (e.g., $\leq 10\ \mu g$) administered isotope tracer is needed to effect a measurable change in the isotopic abundances within physiological lead compartments of an organism (e.g., human), study-dependent increases in lead exposure or perturbation of the size of the inherent lead pools are minimal. The use of stable lead isotope tracer techniques also circumvents the health risks associated with the use of radioactive lead isotopes (e.g., ^{210}Pb, ^{203}Pb), which are now generally precluded from use in human subjects.

The advantages of lead concentration and isotopic composition measurements by TIMS have established an important niche for this technique in research. This has occurred in spite of the relatively high costs of the instrumentation and supplies, expertise required for analyses and equipment maintenance, and the time required for TIMS measurements (see discussions in the isotope dilution and the isotopic composition measurement sections following). Coincidentally, measurements by TIMS have benefited from recent advances that have substantially lowered the costs of the instrumentation, dramatically simplified the analyses, and greatly accelerated the rate of analysis (e.g., the development of multicollector instruments and multisample turrets).

Inductively Coupled Plasma-Mass Spectrometry (ICPMS). A major advance in analyses of both lead concentrations and isotopic compositions has been the development of ICPMS. Since the first commercial instrument was introduced in 1983 (Houk and Thompson 1988), this method has rapidly assumed a prominent position in many research laboratories (e.g., Barnes 1991; Hieftje and Vickers 1989). Inductively coupled plasma-mass spectrometers, in which the sample is introduced into a plasma with excitation temperatures (>5000°K) that efficiently atomize and ionize lead into a mass spectrometer (quadrapole or magnetic sector), are becoming relatively inexpensive and efficient alternatives to TIMS and other established methods (AAS, XRFA, ASV, ICP-AES) (Barnes 1991; Delves and Campbell 1988).

Although ICPMS has just recently become an established method, it is now recognized as potentially the most sensitive multielemental method. It is now beginning to provide widespread, relatively rapid and inexpensive analyses of lead in laboratories using routine procedures (Date and Gray 1989) including, most notably, medical clinics. Additionally, recent studies have demonstrated novel applications of ICPMS (e.g., Brown et al. 1994; Crews et al. 1989; Gercken and Barnes 1991; Owen et al. 1992), which will undoubtedly become more routine in the near future.

There are numerous potential applications of ICPMS in medical and environmental toxicology research (Barnes 1991; Dalgarno et al. 1988; Delves and Campbell 1988). Several novel applications of ICPMS have been made by Barnes and colleagues (e.g., Amarasiriwardena et al. 1992; Barnes 1990; Gercken and Barnes 1991; Lasztity et al. 1989; Viczian et al. 1990) in the analyses of lead and other elements in environmental and biological matrices. For example, liquid chromatography has been coupled with ICPMS to evaluate the biomolecular speciation of lead in human serum (Gercken and Barnes 1991). Other studies have used a suite of elemental tracers (Barnes 1990) and isotope dilution and isotopic composition measurements (Viczian et al. 1990) to evaluate the environmental (soil) sources of childhood lead poisoning.

There have been several reports of relatively good agreement between intercalibrated measurements of blood lead concentrations using ICPMS and other established analytical techniques. These include the early work by Douglas et al. (1983), who found good agreement in analyses using ICPMS and GF-AAS, and by Brown and Pickford (1985), who found comparable results using ICPMS and GF-AAS. Diver et al. (1988) have also investigated the applicability of albumin as a reference material in an intercalibration with ICPMS, AES, and AAS techniques. Subsequently, there have been numerous intercalibrations of elemental concentrations in different biological matrices that compare ICPMS with other techniques (e.g., Beauchemin et al. 1988a,b; Berman et al. 1989; Douglas and Houk 1985; Munro et al. 1986; Pickford and Brown 1986; Ward 1987).

However, there are recognized limitations of ICPMS measurements, as discussed in recent reviews (Barnes 1991; Houk and Thompson 1988; Kawa-

guchi 1988; Koppenaal 1988, 1990; Marshall 1988). The precision and sensitivity of ICPMS measurements (with a quadrapole mass spectrometer) of lead concentrations do not compare to those by TIMS. Additionally, ICPMS has not demonstrated the capacity to produce highly accurate and precise (± <0.05% RSE) measurements of lead isotopic ratios in biological matrices (Delves and Campbell 1988; Russ 1989; Ward 1989) that are routinely produced by TIMS. There are also problems with internal and external calibrations, interference effects, and solids deposition during sample introduction in some ICPMS measurements.

Relatively new innovations in ICPMS instrumentation may partly alleviate some of those limitations. For example, some ICPMS instruments are now available with a magnetic sector mass spectrometer rather than a quadrupole mass spectrometer. These new instruments may provide increased accuracy and precision of lead isotope ratio measurements, which would lead to higher quality measurements by isotope dilution.

Advances in instrumentation may also fill a niche in isotope tracer studies of lead exposure and metabolism in organisms. In those studies, the inherent isotopic abundances are altered by 50% to >200% with the addition of a lead tracer enriched in one of the four stable lead isotopes, and those changes may be readily detected with ICPMS. It is also especially suited for such studies because it can be used to analyze samples relatively rapidly.

Other applications of ICPMS in medical research and public health studies of lead contamination and metabolism would also benefit from additional improvements such as the development of alternative sample introduction techniques, including vapor generation, recirculating nebulizers, ultrasonic nebulizers, and electrochemical furnaces. Benefits would also be gained by improving the sensitivity of ICPMS analyses to the point where nanogram quantities of lead are sufficient to determine isotopic ratios to the limits of the instrument's precision; this may include more sensitive ion detectors, comparable to the Daly detector (Huang et al. 1987) and higher-resolution mass analyzers (e.g., magnetic sector) (Gray 1989). Corresponding reductions in noise and turbulence in the plasma, which may involve the use of other gases for plasma support, will also play an important role in improving isotope ratio measurements (Montasser et al. 1987; Satzger et al. 1987).

Isotope Dilution Mass Spectrometry (IDMS). Lead concentrations are only approximated with any analytical technique, since there is no method that can measure the true concentration of any element in any matrix. The most accurate concentration measurements are made with definitive methods, including isotope dilution mass spectrometry (IDMS) using TIMS. Isotope dilution TIMS is considered the definitive method because it is a yield-independent method of analysis, extremely sensitive, and precise (Webster 1960). The analysis is capable of distinguishing lead from false

signals by simultaneously measuring the relative abundances of its four stable isotopes (^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb) in conjunction with the simultaneous measurements of fragment ions in adjacent masses (e.g., amu 203 and 205). Isotope dilution mass spectrometry methods using TIMS or ICPMS are superior to other measurement methodologies, such as GFAAS and ICP-AES, because the latter are secondary measurement techniques that rely on calibration with secondary standards and standard reference materials (SRMs).

The principles of lead concentration measurements by IDMS are illustrated here. The spike (sp), which is enriched in one isotope (e.g., ^{208}Pb), is added to the sample(s). The $^{208}Pb/^{206}Pb$ isotopic ratio (R) of the homogenized mixture is then measured with a mass spectrometer. The ratio of ^{206}Pb and ^{208}Pb ion intensities in the spiked mass spectrum is equivalent to the sum of the sample portion and the spike portion. The ratio (R) is defined by the following equation, where N is the number of atoms and h is the isotope abundance (%):

$$R = \frac{N_s h_s^2 + N_{sp}^2}{N_s h_s^1 + N_{sp}^1}$$

The number of lead atoms in the sample (N_s) can be determined with the following equation:

$$N_s = \frac{N_{sp}(h_{sp}^2 - Rh_{sp}^1)}{Rh_s^1 - h_s^2}$$

The concentration of lead (e.g., $\mu g/g$) in the sample (G_s) is then determined from the transformation of that equation, in which M is the atomic weight of lead (207.2 amu) and W_s is the sample weight (g):

$$G_s = 1.66 \times 10^{-18} \frac{M}{W_s} N_{sp} \left(\frac{h_{sp}^2 - Rh_{sp}^1}{Rh_s^1 - h_s^2} \right) \qquad [\mu g/g]$$

As evident in the preceding formula, lead concentration measurements by IDMS must also consider the isotopic compositions of lead in the unspiked sample (Huemann 1988). These include natural isotopic variations among samples and isotopic fractionation during the analysis. The latter correction, which is common to all elemental analyses by IDMS, is addressed with standard techniques using certified standard reference materials (e.g., NIST SRM 981). The former correction, which is necessary for few heavy elements besides lead, requries separate isotopic analyses of unspiked samples. This in turn necessitates additional analyses for lead concentration measurements.

Isotopic Composition Measurements. Different sources of industrial lead in the environment, as well as different sources of human exposure to lead, may be characterized and distinguished by their stable lead isotopic

compositions. This approach has been used with great success by a number of investigators in studies of lead cycling in marine (Flegal and Patterson 1983; Flegal and Stukas 1987; Smith et al. 1990, 1992a) and terrestrial (Elias et al. 1982; Rabinowitz and Wetherill 1972) environments, as well as in studies investigating human lead exposure (Manton 1985; Rabinowitz 1987; Smith et al. in manuscript; Tera et al. 1985) and metabolism (Rabinowitz et al. 1973, 1976, 1977; Smith et al. 1994). For example, Rabinowitz (1987) used stable lead isotopic compositions to identify the primary source of lead contamination (lead paint-contaminated soils) in several lead-poisoned children in Boston.

While all the isotopic compositions in these studies were measured with TIMS, other studies have utilized ICPMS measurements of lead isotopic compositions. For example, Dean et al. (1987) measured lead isotopic compositions in milk and wine, while comparable ICPMS analyses of lead isotopic compositions in other environmental matrices have been made by Date and Chung (1987), Delves and Campbell (1988), Caplun et al. (1984), Longerich et al. (1987), and Sturges and Barrie (1987). These studies indicated that quadrupole ICPMS measurements did not provide sufficient precision to adequately distinguish environmental lead sources on the basis of their stable isotopic composition (Russ 1989; Ward 1989) since the isotopic abundances of contaminant environmental lead typically vary by only 3%–7%. Further, most published ICPMS measurements of lead isotopic compositions in environmental and biological matrices have not included ratios for ^{204}Pb (1.4% natural relative abundance), which is required for definitive isotopic composition analyses.

Isotopic composition measurements, as well as lead concentration measurements by IDMS, require a correction for the contaminant lead blank. The correction is particularly important in cases where an enriched isotopic tracer is used. It includes analyses of all contaminant lead added during sample collection, storage, processing, and analysis (Flegal and Smith 1992b; Patterson and Settle 1976). The isotopic composition and amount of each of those contaminant lead additions to the sample must be determined and corrected for separately because the relative contribution of the amount of blank lead (e.g., pg of Pb) to the sample at each stage of processing may be different than the contribution of the blank's isotopic composition to the sample since there may be less than 100% transfer efficiency of sample + blank lead from one processing step to the next. In other words, the amount of blank lead contributed to the sample will be affected by the lead transfer efficiency at each stage, while the isotopic contribution of that blank will be affected by both the transfer efficiency and isotopic composition of the blank at each processing step. For comparison, typical lead blanks from containers and reagents used for blood and serum lead analyses in our trace metal clean laboratory are listed in Table 1.

Accurate corrections for contaminant lead blanks are essential for producing high quality data in studies utilizing administered stable isotope

tracers. The influence of a constant blank amount and isotopic composition on the sample isotopic composition increases nonlinearly with increasing isotopic differences between the sample and blank. Sample versus blank isotopic differences, while normally relatively small (<5%) in environmental samples, can be very large (>200%) in stable lead isotope tracer studies. Large differences result in significant blank corrections in cases where the blank lead content is >5% of the sample lead. In those cases, the accuracy and precision of the sample and the blank lead content measurements will determine the accuracy of the true blank corrected sample isotopic composition (assuming isotopic ratio measurement errors are <0.05% RSE). In our studies, the isotopic correction of a sample for a contaminant blank is carried out using the general formula

$$(A/B)_c = \frac{(A/B)_m - ((A/B)_b{*}X)}{1 - X},$$

where $(A/B)_c$ = blank corrected ratio of isotopes A and B in the sample, $(A/B)_m$ = measured ratio of isotopes A and B in the sample, $(A/B)_b$ = measured ratio of isotopes A and B in the blank, $X = (B_b/B_m)$, B_b = abundance of isotope B in the blank, and B_m = abundance of isotope B in the sample.

The propagated contribution of lead concentration and isotopic ratio measurement errors (Bevington 1969) to the final isotopic composition error values may be determined with the formula

$$\sigma_c = \frac{\left\{(\sigma_m)^2 + (\sigma_b{*}X)^2 + \left[\sigma_x{*}\left(\frac{b - m}{1 - X}\right)\right]^2\right\}^{1/2}}{1 - X},$$

where σ = standard error of the mean, σ_c = corrected error on corrected isotopic ratio [$(A/B)_c$ above], σ_m = measured error on measured isotopic ratio, σ_b = measured error on blank isotopic ratio, σ_x = error on measurement of 'X' term, m = isotopic ratio of measured sample, b = isotopic ratio of blank, X = same as in previous equation.

K. *In Situ* Measurement Methodologies

A number of techniques are available, including laser microprobe mass analysis (LAMMA) and various microprobes, that are capable of determining within tissues or cell preparations the cellular and subcellular distribution of lead *in situ*. While these techniques generally require varying levels of sample preparation (e.g., tissue fixation, cell suspensions, etc.), they provide powerful tools for probing different cellular compartments and processes involving lead. Thus, the site-specific distribution of lead can be used to evaluate local toxicity, which can be correlated with pathological alterations in tissues.

LAMMA. Laser microprobe mass analysis was specifically developed to complement other microanalytical techniques to determine intracellular distributions of physiological cations and toxic constituents in biological tissues. LAMMA is an analytical methodology capable of simultaneous multielement analysis of metals and their distribution on a cellular or subcellular scale, with a measurement sensitivity of 10^{-17} to 10^{-20} g for most metals. LAMMA has received somewhat widespread use in the analysis of essential and toxic metals in tissues and cell preparations (Drüeke 1980; Goebel et al. 1990; Schmidt and Barckhaus 1991; Schmidt et al. 1980, 1986; Vandeputte et al. 1985; Verbueken et al. 1984; Visser et al. 1984). The detection sensitivity of LAMMA for lead is 5 μg/g, with a limit of detection of approximately 2×10^{-18} g (Schmidt and Barckhaus 1991).

Studies using LAMMA have demonstrated its utility as a method to investigate the cellular distribution of lead within various cell and tissue types. Schmidt et al. (1985) studied the morphological distribution of lead within substructure microsamples (1–3 mm) of the vascular wall. The topochemical distribution of lead in the human arterial wall was determined using LAMMA (Linton et al. 1985; Schmidt et al. 1985), as was the localization of lead in different cell types of bone marrow of a lead-poisoned individual (Schmidt and Ilsemann 1984) and the distribution of lead in placental tissue and fetal liver after acute maternal lead intoxication (Schmidt et al. 1980).

However, LAMMA is a sample-destructive technique, and quantification of the local ion concentrations is still under investigation. While it has often been assumed that the inherent metal binding/distribution of the elements under study is not altered by processing and analysis with LAMMA, few studies have systematically investigated the loss or addition (i.e., contamination) of elements during tissue processing for microanalysis (Blaineau et al. 1988; Lane and Martin 1982; Morgan et al. 1975; Vandeputte et al. 1985, 1990). Vandeputte et al. (1990) observed that lead-induced inclusion bodies in rat kidney tissue fixed with glutaraldehyde and OsO_4 contained large amounts of calcium but no measurable amounts of lead. A similar observation was made by Zhong et al. (1987) in a study using the electron microprobe. It was determined that postfixation of glutaraldehyde-fixed tissue with OsO_4 caused a notable decrease in the LAMMA-detected lead signal, suggesting some modification of the protein binding of lead within the inclusions (Vandeputte et al. 1990). Based upon these observations, sample preparation using cryotechniques (snap-freezing and vacuum drying), rather than fixative procedures, may be preferable to minimize the possible loss or contamination of lead within the sample.

Microprobes (Electron, Proton, Photon). Most microprobe systems are based on the principle that excited elements within the sample emit characteristic fluorescence x-rays. For electron- and proton-based microprobes, characteristic fluorescence x-rays are generated from elements in the sample

by an electron or proton beam that is focused by magnetic fields. The beam can be scanned over a sample and the x-ray spectrum from specific locations recorded by a detector, thereby generating a two-dimensional image of the sample's elemental composition. Photon-based microprobes using either x-ray or gamma-ray beams are used in a similar fashion, but the photon beam is generally focused by means of collimating and shielding a wider signal because photons are neutral. The focusing of charged beams (e.g., electrons and protons) is much easier, and these usually provide better spatial resolution than photon sources. Following is a brief discussion of some of the most common microprobe techniques.

Electron X-ray Microprobe. X-ray microprobe measurements have been used to identify regions of relatively high metal concentrations at the cellular and intracellular level. For example, electron x-ray microprobes have been employed to identify lead-rich cytosolic and nuclear inclusion bodies in rat renal tubule cells following elevated exposures to lead (Fowler et al. 1980; Oskarsson and Johansson 1987).

PIXE. Proton-induced x-ray emission (PIXE) is a relatively nondestructive technique with detection limits in the parts per million range (Torok and Van Grieken 1994). It has been used in studies of the microdistribution of toxic metals (e.g., lead) in thin-sliced sections of tissues (Lindh et al. 1978; Lowe et al. 1993; Schidlovsky et al. 1990). The simultaneous quantification of a large number of elements in a sample makes PIXE an important analytical tool for use in diverse toxicological studies (Johansson and Campbell 1988). Those applications were recently discussed in a report on elemental analysis of renal slices (Lowe et al. 1993). However, the shallow sampling depth (tens of microns) of the proton beam restricts applications of PIXE to measurements of surface distributions.

SRIXE. Synchrotron radiation-induced x-ray emission (SRIXE) utilizes a synchrotron radiation x-ray source to induce the emission of characteristic fluorescence x-rays. Synchrotrons are capable of producing extremely high-intensity pulsed beams of x-rays which allow the detection of low levels of trace elements, including lead. Spatial resolution is typically in the tens of millimeters, although this may be improved to about 1 mm (Jones and Gordon 1989). An apparent advantage of SRIXE over other microprobe sources is that the damage to the analyzed sample caused by the photon beam is estimated to be at least an order of magnitude less than the damage caused by the charged particles of proton and electron beams (Slatkin et al. 1984). The lower sample damage from radiation in SRIXE may be important for the analysis of living tissues and cells in which the preservation of tissue/cell integrity and metal distribution is a primary concern. Because there are only a few synchrotrons, there are only a few laboratories that can make SRIXE measurements using x-rays in the keV energy range.

EXAFS and XANES. Extended x-ray absorption fine structure (EXAFS) and x-ray absorption near edge structure (XANES) are two other types of x-ray spectroscopy, both of which utilize synchrotron radiation to investigate structural and speciation information in an atomic environment within a prepared sample target (Gordon and Jones 1991). These techniques may complement other analytical techniques (e.g., x-ray microprobe) to provide important additional information on the chemical structure of lead complexes at a spatial resolution of 1 mm^2 and detection limits in the 5-ppm range. However, applications of this technique in studies to quantitate speciation generally require a comparison of the sample absorption spectrum with those of model compounds that contain the absorbing atoms of interest, including the range of its oxidation states and types of ligands (Gordon and Jones 1991). Again, there are only a few facilities with capabilities for these types of measurements.

L. Hybrid Analytical Techniques

Coupled, or 'hybrid,' instrumentation techniques are analytical methodologies involving the interfacing of two or more established analytical techniques, such as a subcellular or biomolecular separation technique coupled with an analyte detector. Coupled instrumentation has been developed during the past 15 yr in part to address some of the difficulties in determining the biomolecular speciation of metals, including lead. Several of these difficulties are based upon recognized limitations in the use of exogenous (i.e., noninherent) tracers of metal metabolism and speciation, such as radioisotopes. Among these is the difficulty in determining whether the tracer is taking part in all the reactions contributing to the distribution of the metal without altering those reactions. The relatively lengthy (i.e., > hours) separation and analysis schemes required in many current methods may also contribute to an alteration of the inherent binding of the metal–biomolecule complex (e.g., Borguet et al. 1990; DuVal et al. in manuscript). Coupled analytical methodologies may circumvent many of these concerns by offering excellent analytical sensitivity and the capability of on-line separation/analysis of inherent or endogenously bound metal species (Barnes 1991; Crews et al. 1989; Ebdon and Hill 1989).

High-performance liquid chromatography is the most common chromatographic method for the separation of lead biomolecules in complex samples. As previously noted, there are several available HPLC separation methods (e.g., size exclusion, ion-exchange, normal, and reversed-phase) that are all suitable for use with atomic absorption or mass spectrometry (Barnes 1991; Cleland et al. 1994; Ebdon et al. 1987; Gercken and Barnes 1991; Owen et al. 1992; Tomlinson et al. 1994; Uden 1989). Analyte detectors in coupled instrument configurations may be single (e.g., AAS) or multielement (e.g., ICPMS) in design (Barnes 1991; Ebdon et al. 1986, 1987; Uden 1989).

ICPMS coupled with a suitable chromatographic methodology has

shown particular promise for a number of applications in metal speciation studies (Barnes 1991; Brown et al. 1994; Bushee 1988; Bushee et al. 1990; Crews et al. 1989; Hietkemper et al. 1989). The most important advantages of ICPMS in these systems are its (i) high elemental sensitivity and selectivity, with detection limits comparable to GFAAS (i.e., sub-ppb); (ii) ability to monitor elemental concentrations directly in the eluent of the chromatographic system; (iii) capacity to measure several elements simultaneously; and (iv) capabilities to measure stable isotopic compositions (Barnes 1991; Dean et al. 1987; Delves and Campbell 1988; Gercken and Barnes 1991; Paschal 1990). Studies using size exclusion chromatography coupled with ICPMS detection have investigated several protein-bound metals, including lead and cadmium (Brown et al. 1994; Crews et al. 1989; Dean et al. 1987; Gercken and Barnes 1991; Owen et al. 1992).

A potentially valuable, though relatively unexplored, application of chromatography coupled with ICPMS is in stable isotope tracer studies. Stable isotope tracer methodologies have proved to be powerful techniques for investigating the metabolism of lead and other nephrotoxic metals at low dose exposures, as previously noted (e.g., Smith and Flegal 1992a; Smith et al. 1992b, 1994). Studies of metals (e.g., lead) possessing multiple measurable stable isotopes may be used to evaluate several factors simultaneously, such as the temporal effects of exposure on lead speciation or the differences in intrinsic versus extrinsic tracer labeling.

VI. Measurements of Human Lead Exposure and Toxicity

Contemporary humans are exposed to elevated levels of lead through essentially every aspect of their existence, including lead-contaminated aerosols, dusts and soils, water, and food (Flegal et al. 1990; USEPA 1986). Of the approximately 10–200 μg of lead ingested daily by adults (Flegal et al. 1990; USEPA 1986), only 5%–20% is absorbed from the gastrointestinal tract (Fig. 9). About half of the absorbed lead is subsequently excreted via the kidneys and bile ($t_{1/2} \approx 20$ d), while much of the remainder is distributed to peripheral soft tissues and the skeleton (Rabinowitz et al. 1976). The body burden of lead in humans is contained primarily ($>90\%$) in the skeleton, which is composed of long-lived compartments of cortical (elimination half-life >5–10 yr) and trabecular (elimination half-life ≈ 1 yr) bone, with comparatively small amounts of lead in compartments that are in rapid exchange with extracellular fluid and plasma (Hryhorczuk et al. 1985; Manton 1985; Marcus 1985; O'Flaherty 1991; Rabinowitz et al. 1977; Schutz et al. 1974). Consequently, the skeleton is believed to best reflect a time-integrated exposure of the individual (Barry 1975; Patterson et al. 1991; Wittmers et al. 1988).

A. Measurements of Lead Exposure

Ideally, markers of human lead exposure should reflect past and current exposures of both a chronic and an acute nature. Realistically, no single

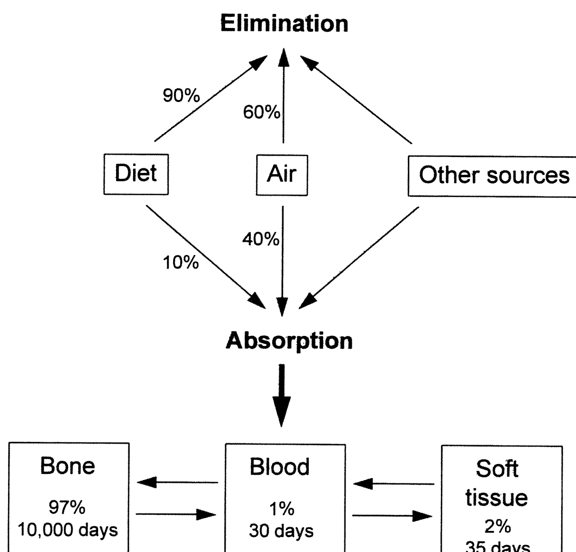

Fig. 9. Lead absorption in humans. Numbers in the physiologic compartment boxes are the relative percentage (%) of the body burden and the biological mean-life of lead in that compartment. (Data from Rabinowitz et al. 1973, 1976, 1977.)

marker is capable of this, because of the complex toxicokinetics of lead relating exposure to disposition within organisms. Measurements of blood lead levels are still regarded as the best exposure marker available (NRC 1993), although they are limited because blood lead levels primarily reflect only relatively recent (< months) exposures (Marcus 1985; Rabinowitz et al. 1973, 1976) (see Fig. 9). *In vivo* XRF measurements of bone lead levels have proved very useful in providing some integrated measure of past exposures that have occurred over longer (i.e., yr) intervals, particularly in cases of occupational exposures (Ahlgren et al. 1976; Christoffersson et al. 1984; Somervaille et al. 1988; Todd and Chettle 1994; Watanabe et al. 1994). Other indicators of exposure include measurements of lead in teeth, which also provide an integrated measure of long-term exposure. Conversely, measurements of lead in urine, hair, and nails have proved less useful because of (i) their large within-subject variability (urine), (ii) their susceptibility to contamination (urine, hair, nails), and (iii) the inadequately defined relationships between exposure and sample lead concentration (hair and nails).

B. Stable Lead Isotopic Tracers in Metabolic Studies

Stable lead isotope tracer methodologies have provided the most definitive measurements of lead metabolism in humans (Manton 1985; Rabinowitz et al. 1973, 1976, 1977; Smith et al. 1994). The inherent precision ($\leq 0.05\%$ RSE) and sensitivity (<1 ng sample Pb) of this technique allows the use of

very low exposure levels in lead tracer studies. This technique overcomes many of the analytical (poor precision and sensitivity) and health (exposure to radioactivity) limitations encountered in studies employing simple lead concentration measurements or radiolead isotopes. However, stable lead isotope studies still require trace-metal-clean procedures to control incidental sources of lead that may confound interpretation of the results (Patterson and Settle 1976; Smith and Flegal 1992a; Smith et al. 1992b).

Several recent studies have demonstrated the utility of ultraclean stable lead isotope techniques using TIMS to investigate skeletal and soft tissue lead metabolism during homeostasis as well as during treatment with therapeutic chelating agents in laboratory animals and humans (Smith and Flegal 1992a; Smith et al. 1992b, 1994, in manuscript). These studies evidence the minor error inherent in TIMS stable isotope tracer techniques relative to other sources of error (e.g., biological variability). Again, the new generation of magnetic sector ICPMS instruments may prove to be more suitable for tracer applications than previous quadrupole ICPMS instruments.

These advances in stable istopic tracer measurements are important because of the limitations in lead radiotracer measurements. Tracer studies of lead metabolism using radioisotopes (^{203}Pb, ^{210}Pb, and ^{212}Pb) have been hindered by the relatively poor precision of the measurements, their relatively short (^{203}Pb $t_{1/2}$ = 51.88 hr and ^{212}Pb $t_{1/2}$ = 10.64 hr) or long (^{204}Pb $t_{1/2}$ = 22.3 yr) half-lives, and the relatively weak gamma energy of ^{210}Pb. Additionally, concerns about the radiation hazard of these isotopes have severely restricted their utilization in many laboratory and clinical studies.

C. Biological Markers of Lead Toxicity

Elucidation of some of the mechanisms of lead toxicity on the cellular and biochemical level has led to the development of several relatively sensitive biomarkers of lead exposure and toxicity, including measurements of the effects of lead on enzymes of the hematopoietic system. Lead-induced alterations in blood zinc protoporphyrin (ZPP) and erythrocyte δ-aminolevulinic acid dehydratase (ALAD) activity have been established as relatively specific biomarkers of lead toxicity to the heme biosynthetic pathway (NRC 1993; USEPA 1986). Increases in blood ZPP occur as a result of inhibition of ferrochelatase (FC) by lead. Inhibition of ALAD by lead, which begins at a blood lead level of about 5 μg/dL (Chisolm et al. 1985; USEPA 1986), is considered to be one of the most sensitive biomarkers currently available.

Although ZPP accumulation in erythrocytes is directly correlated with blood lead levels (Alessio 1988; Chisolm and Barltrop 1979; Piomelli et al. 1982), the response threshold (15–25 μg/dL) is above the level at which neurobehavioral toxicity occurs in children (Bellinger et al. 1987, 1991; Dietrich et al. 1992, 1993a,b; McMichael et al. 1988; Needleman et al. 1979, 1990; Schwartz 1994; Schwartz and Otto, 1987; Wasserman et al. 1994). The use of increased ZPP levels in lead screening of high-risk populations

also appears to yield an increasingly unacceptable rate of false-negative results as both population blood concentrations and the guideline concentrations for what is an acceptable exposure decline (Mahaffey and Annest 1986; CDC 1991; NRC 1993). Consequently, the recent CDC (1991) statement for childhood lead poisoning no longer contains ZPP measurements as a criterion in diagnosing childhood lead intoxication.

Other biomarkers of lead exposure and toxicity include (i) the development of metal-specific porphyrinuria patterns involving aminolevulinic acid (ALA) and other porphyrin isomers (Fowler and Mahaffey 1978; Mahaffey et al. 1981; NRC 1993; Silbergeld and Fowler, 1988; USEPA 1986); (ii) the inhibition of the enzyme pyrimidine-5′-nucleotidase, which catalyzes the hydrolysis of pyrimidine nucleotides from degraded ribosomal RNA fragments in maturing erythrocytes (Angle and McIntire 1978; Paglia and Valentine 1975); and (iii) the presence of specific lead-binding proteins (PbBP) in target tissues or urine (Church et al. 1993; Fowler and DuVal 1991; Goering and Fowler 1987; Mistry et al. 1985; Oskarsson et al. 1982; Shelton and Egle 1982). Continued development in these areas is expected to provide a framework for understanding why individuals vary in their susceptibility to measurable toxicity.

D. Biomolecular Speciation of Lead

Knowledge of the speciation of lead in whole blood and plasma, as well as in other tissues, over a range of exposures should provide a better understanding of the toxicokinetics of lead. Many of the determinations of lead speciation have been limited by contamination problems associated with measuring low lead concentrations (<3 ng/mL), as well as the limitations of techniques to evaluate the chemical species of lead present. Procedural difficulties in evaluating the toxic effects of lead at the cellular level cannot be overestimated since serum lead concentrations are generally <1% of blood lead levels, while free Pb^{2+} in serum and within cells has been estimated at 10^{-12} to 10^{-11} *M* (Al-Modhefer et al. 1991; Schanne et al. 1989b; Simons 1993). Nonetheless, studies are needed to establish the relationships between specific biological responses to lead in target tissues and the inorganic or molecular chemical species that contribute to the response.

Lead–protein interactions may serve as a basis for many of the mechanisms that underlie lead toxicity (see Goering 1993 for a recent review). Lead has been shown to interact with numerous proteins and enzymes within target tissues, including ALAD, ALA synthetase, and FC of the heme biosynthetic pathway (Fowler et al. 1980; Silbergeld and Fowler 1988), cytosolic high-affinity lead-binding proteins in kidney and brain (Goering and Fowler 1987; Mistry et al. 1985; Oskarsson et al. 1982; Shelton and Egle 1982), metallothioneins (Church et al. 1993; Goering and Fowler 1987), and calcium signal transduction proteins such as calmodulin and protein kinase C (Long et al. 1994; Markovac and Goldstein 1988).

Data from these and other studies have been invaluable in the development and testing of hypotheses on the role of lead-binding proteins in mediating target cell/tissue toxicity, including alterations in renal proximal tubule cell gene expression (Fowler 1992; Fowler and DuVal 1991; Fowler et al. 1993, 1994).

VII. Conclusions

In conclusion, accurate and precise measurements of lead, including environmental and human lead burdens relative to natural levels, lead cycling and metabolism, and lead biomolecular speciation, are required to fully evaluate the problem of lead contamination and toxicity in the biosphere. Current data demonstrate that environmental lead levels are often more than 10-fold and sometimes more than 10,000-fold higher than natural levels. Although regulation of many lead-containing products in some industrialized countries has resulted in a reduction in some lead discharges into the biosphere, the demonstrated persistence of contaminant lead indicates that environmental lead levels will remain substantially elevated for a protracted time.

Lead-contaminated environments have resulted in comparable increases in organism and human lead burdens, as indicated by a recent estimate of the natural level of lead in blood of preindustrial humans (0.016 μg/dL or 0.8 nM). This estimate has important public health implications because it suggests that blood lead levels that are now considered acceptable in children (i.e., <10 ug/dL or <480 nM) are nearly 600-fold greater than estimated natural levels, while they are only ≈ 10-fold lower than levels (≈ 100 μg.dL or 4800 nM) that may cause encephalopathy and death in many individuals of a population. Understanding of the extent of sublethal lead toxicity in humans may benefit from studies that consider control populations possessing natural (i.e., preindustrial) lead burdens.

Summary

The importance of accurate measurements of environmental lead exposure and toxicity is substantiated by analyses documenting the global contamination of the biosphere with industrial lead and the pervasiveness of measurable lead toxicity in human populations. Those data demonstrating environmental lead contamination and toxicity have, in part, led to regulations that limit the amount of lead in some products (e.g., paint, solder, and gasolines) in many industrialized countries. These regulations have resulted in a substantial reduction in some lead discharges to the environment. In spite of these reductions, current environmental lead levels are still often more than 10-fold, and sometimes more than 10,000-fold, higher than natural levels. Further, environmental lead concentrations are expected to re-

main elevated for a protracted period due to continued emissions of relatively large amounts of industrial lead to the environment and the persistence of contaminant lead in the environment.

Discharges of contaminant lead have resulted in increases in organism and human lead levels comparable to increases documented in environmental matrices, as indicated by a recent estimate of the natural level of lead in blood of preindustrial humans (0.016 μg/dL or 0.8 nM). This estimate is 175-fold lower than average blood lead levels in the United States (2.8 μg/dL or 140 nM) and 600-fold lower than the recently (1991) revised Centers for Disease Control (CDC) action level of concern for early toxic effects in children (10 μg/dL or 480 nM). The significance of these comparisons to public health is corroborated by numerous studies suggesting that there may be no lower threshold for sublethal toxicity in contemporary (i.e., lead-contaminated) humans. Those data also indicate that environmental lead concentrations that were previously considered innocuous may be deleterious to human health.

It is apparent that the extent of sublethal lead toxicity in humans may be best addressed by studies that consider control populations possessing natural (i.e., preindustrial) lead burdens, as well as state-of-the-art, trace-metal-clean techniques and advanced instrumentation. Trace-metal-clean techniques are required to prevent the inadvertent lead contamination of samples, which has plagued many previous analyses of environmental and human lead levels. Advanced instrumentation is required to provide the sentivity, accuracy, and precision that are needed to quantify the sublethal effects of lead concentrations at environmental levels of exposure. Fortunately, methodologies utilizing these advancements are now capable of addressing many of the important issues (e.g., lead biomolecular speciation, low exposure effects) in environmental and human lead toxicology.

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Epidemiology of Atrazine

Rolf Loosli*

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I. Introduction

Herbicidal *s*-triazines have been on the market for more than 35 years; simazine was introduced in 1956 and atrazine in 1957 (Worthing 1991). The current list contains a dozen important compounds (Loosli 1994). Experimental toxicology data suggested *s*-triazines to be safe in production and in recommended applications to crops. Health data of manufacturers and field workers corroborated the favorable expectations; that is, factories and farms have never reported health effects in personnel.

The perception of triazines as "safe compounds" was modified when a chronic study conducted in the mid-1980s showed effects of atrazine on mammary tumors in rats (WHO 1990). As a result, manufacturers introduced restricted personnel exposure levels. The discovery that atrazine was often present where pesticide residues in drinking water exceeded the newly defined European limit of 0.1 μg/L for any active ingredient, and subsequent media reports and public discussions that made no distinction between "detection" and "hazard," promoted a feeling of insecurity toward the formerly unchallenged products. The development reached a level of misinformation such that atrazine was occasionally chosen as an instrument to attempt suicide (Section III). A virtually unlimited level of tolerance was fortunately experienced in these unfortunate attempts.

To bypass interspecies differences that handicap human hazard estimates based on animal toxicity data, several authors have in recent years investi-

*Rebhaldenstr. 3, CH-4411 Seltisberg, Switzerland.

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gated the health data of cohorts and individuals exposed to *s*-triazines. Because of the long history of the products, that is, the long lag between early exposures and evaluation, such studies are a valid tool to detect even delayed chronic effects with a long latency. This review assesses data on manufacturing cohorts and rural populations as well as case reports of acute exposure of humans and domestic animals. The prevalence of atrazine in the reports reflects its wide distribution. Sporadic reports specifying simazine and other analogs were included to expand the database.

II. Epidemiology of Chronic Exposure Effects in Humans

A. Studies in Rural Populations and Field Workers

In a case-control study in northern Italy, all 66 cases of primary ovarian tumors diagnosed in 6.5 yr in a city hospital were analyzed (Donna et al. 1984). The history of herbicide exposure was established by interview in 60 cases; 6 remained untraced, and 127 controls were cases of cancer in other organs from the same hospital. The cases were matched by year of diagnosis, age, and district of residence. Personal herbicide use was reported for 8 cases and 0 controls, and probable exposure (employment on farms, residence in areas of herbicide use) for 10 cases and 14 controls. Relative risk (RR) for ovarian tumors associated with herbicide exposure was 4.4 [95% confidence interval (CI) 1.9–16.1]. For women under 55 years, the RR rose to 9.1 (CI, 3.0–28.2).

In a subsequent study, the same authors interviewed 65 histologically confirmed primary malignant epithelial ovary tumor cases diagnosed in a 5-yr period (Donna et al. 1989). Two controls per case, matched for age, were randomly selected from regional electoral rolls. Seven cases and 7 controls reporting definite contact gave an odds ratio (OR) of 2.7 (90% CI, 1.0–6.9). Possible contact was reported by 14 cases and 20 controls, giving an OR of 1.8 (90% CI, 0.9–3.5). Definite exposure for more than 10 yr showed a slightly higher OR than exposure for less than 10 yr (2.9; 0.1–8.7 vs 2.3; 0.4–12.3).

A reviewer challenged the results on three grounds: the statistics suggest that the results could be due to chance, the exposure classification considers use of triazines and work in treated fields as equivalent, and there are various sources of bias (Minder 1990). The 90% confidence limits used by the authors reflected a weak significance. The data suggested that additional work may be warranted but do not support any definite conclusion. The authors, however, maintained the adequacy of their statistical and interviewing methods and believe that triazines as stable chemicals lead to substantial exposure during work carried out in fields where the herbicides are used (Crosignani et al. 1990).

The International Agency for Research on Cancer (IARC), in its monograph on atrazine (IARC 1991), further reviewed five case-control studies concerning farming populations in the United States. No association of

atrazine with leukemia was demonstrable in Iowa or Minnesota (Brown et al. 1990). Small-cell lymphocytic lymphoma showed a slightly elevated OR of 1.6 (Cantor et al. 1985). For non-Hodgkin's lymphoma (NHL), in Kansas an OR of 2.5 resulted from exposure to six triazines including atrazine, together with other herbicides; for triazines alone, the OR was 2.2 on the basis of 3 cases and 11 controls (Hoar et al. 1986). In Nebraska (Hoar Zahm et al. 1988, 1990), the OR rose from less than 1 for 1–15 yr of atrazine use to 2.0 for 16 yr or more. Another study in Kansas indicated a slight excess risk of colon cancer (OR, 1.4) with use of triazines (Hoar et al. 1985).

The possibility of an association of NHL with triazine exposure was further assessed using data from four midwestern U.S. states (Hoar Zahm et al. 1993). Three case-referent studies involved 993 NHL cases and 2918 population-based referents. Interviews concerning the agricultural practices of persons in the study yielded an age-adjusted OR of 1.4 for patients who reported that atrazine was used on the farm where they lived or worked. The OR was the same when the analyses were restricted to persons who had personally handled atrazine. Adjustment for the use of other pesticides reduced most of the calculated associations between NHL and atrazine, especially those of the long-term and frequent users in a detailed analysis. The data suggested no consistent increase in the risk of NHL attributable to the agricultural use of atrazine.

B. Workers in the Chemical Industry

Whereas the agricultural use of atrazine is usually seasonal, production workers and formulators in chemical plants are often in contact with the same substance at higher concentrations year-round. Epidemiology studies on industrial cohorts therefore deserve special consideration.

The morbidity of triazine factory workers was analyzed in a historic-prospective study that involved 154 matched worker pairs in a Swiss plant (Gass 1991). There was no difference in clinical parameters between workers in the triazine area and in other areas of the same plant. The health histories of the cohort compared favorably with those of the nonexposed controls, with the exception of gastritis/gastroenteritis, which had a significantly ($p < 0.001$) higher incidence in the atrazine cohort. Confounding variables, including tobacco and alcohol as well as the duration of exposure, did not affect the difference. The author considered the possibility of a causal relationship between triazine exposure and gastrointestinal effects but reached no definite conclusion.

A clinical review based on details of the gastrointestinal case histories eliminated 11 of 20 cases because they were clinically acute. Of the remaining 9 chronic cases, 2 were gastric ulcers; the remainder represented a variety of descriptive diagnoses with heterogeneous pathology (Stalder GA, unpublished letter). There was no correlation with the duration of exposure. No such incidence of gastrointestinal effects was recorded among the

personnel of plants manufacturing triazines in the United States (Ciba-Geigy 1991, personal communication). The incidence is therefore considered unrelated to triazines.

Retrospective studies evaluated the mortality experience in two cohorts of chemical workers in an Alabama and a Louisiana manufacturing plant (Delzell and Sathiakumar 1992; Sathiakumar et al. 1992). Data from the two studies were pooled to increase the database and thus enhance the informativeness of the results. The 2683 workers with definite or probable exposure to triazines, especially the long-term employees, had a lower mortality rate than the general population (healthy worker effect). The mortality rate in 2234 possibly exposed workers was similar to that of the general population.

The only difference in specific syndromes was an excess of 3 observed versus 0.8 expected NHL cases in the "definite or probable" group. Job histories of the NHL subjects did not, however, reflect shared exposure; two of the men had spent only 42 d in triazine areas. It is therefore unlikely that there is any causal relationship between triazine exposure and NHL. The fact that NHL was not observed in the Swiss cohort (Gass 1991) adds further weight to this conclusion.

III. Sporadic Cases of Acute/Subacute Poisoning in Humans

In the practical experience of manufacturers and applicators, herbicidal *s*-triazines have proved to be virtually nontoxic. Consistent evidence of toxic effects has not been reported (Deutsche Forschungsgemeinschaft 1986; Loosli 1994; Velvart 1993). It is particularly pertinent that the health files of major manufacturing plants have no record of triazine poisoning, although safety precautions in the early years of production prevented worker exposure to a limited extent only (Ciba-Geigy 1993, personal communication).

Three cases of attempted intentional poisoning with products containing atrazine were communicated to a manufacturer in unpublished letters by attending physicians (Ciba-Geigy 1993, personal communication):

- An adult male swallowed an estimated 1000 g of a 50% wettable powder (WP) formulation. The man vomited and was found in a worried state. His family took him to an emergency facility where atropine (therapy for phosphoric ester insecticide poisoning) was administered. The patient was then transferred to the hospital where he was successfully treated for atropine poisoning. The hospital diagnosed no other signs requiring therapy.
- A woman administered to her 6-yr-old child and to herself undefined amounts of two products, one based on atrazine and the other on barium silicofluoride. The mother was found unconscious and recovered under intensive care. Her serious condition was attributed to the highly toxic

barium derivative. There was no evidence of triazine poisoning. The child was not clinically affected.

- A farmer swallowed 2 L of an aqueous formulation containing 50% atrazine and 5% ethylene glycol. The man developed serious signs of ethylene glycol poisoning. While specific therapy was given to control this emergency, the hospital reported no complicating signs suggesting atrazine effects.

A case of fatal poisoning was ascribed to ingestion of 500 mL of a product containing 100 g atrazine, 25 g aminotriazole, 25 g ethylene glycol, and 0.15 g formaldehyde (Pommery et al. 1993). The patient was hospitalized in a state of coma and was treated for severe ethylene glycol poisoning. He died after 3 d from intractable shock; autopsy further showed necrotic enteritis and hemorrhagic rhabdomyolysis. The authors considered the clinical evolution not typical of ethylene glycol effects and speculated that the herbicidal components, and possibly formaldehyde, determined the fatal course of the case.

A possible case of subchronic herbicide poisoning was seen in a diabetic patient with a history of past alcoholism (Gallois and Dereux 1979). The man was engaged in railroad track weed control, where he manually applied a product containing atrazine, tebuthiuron, and aminotriazole. During the campaign, he was hospitalized in a confused state with sporadic convulsions and loss of consciousness. He showed signs of peripheral neurogenic effects of the legs, gastrointestinal effects, and elevated protein level of the spinal fluid. Electromyography showed slow motoric conduction. The authors speculated that the gastrointestinal effect was associated with tebuthiuron and the peripheral neurogenic signs with atrazine. They considered diabetic neuropathy less likely because within 2 months the patient recovered clinically without specific therapy.

IV. Acute Triazine Poisoning in Ruminants

Experimental toxicity trials showed cattle and sheep to be less tolerant than rodents to oral intake of triazines and other herbicides (Milhaud and Pilault 1977). One dose of 500 mg/kg simazine had a lethal effect in sheep (Hapke 1986). Toxic effects cumulated with successive doses: 31 daily doses of 50 mg/kg or 14 doses of 100 mg/kg were lethal to sheep. Corresponding results were obtained with atrazine and prometone (Palmer and Radeleff 1969). Signs of severe poisoning were weakness, ataxia, and posterior paresis or paralysis, appearing with a lag of as much as 3 weeks. Substances were administered by capsules or drench.

To simulate natural grazing conditions, cattle and sheep were fed during 25–27 d on hay that before cutting had been sprayed with atrazine or prometone at rates of 22.5 and 44.9 kg/ha, respectively. The triazine intake was highest in week 2, when consumption in sheep and calves reached 68

mg/kg of body weight (bw) for atrazine, or 75 mg/kg bw for prometone. Clinical parameters remained normal throughout the test; no histopathological changes attributable to treatment were detected. Body weight development in the atrazine groups was regular but in the prometone groups was inferior to controls presumably because of palatability problems (Johnson et al. 1972).

To test the therapeutic effect of activated charcoal as an adsorbent following accidental ingestion of large doses, atrazine 400 mg/kg bw was administered to cattle by gavage in a single dose (Kobel et al. 1985). Three animals were given charcoal, 454 g per animal, at 4, 24, 48, and 72 hr after dosing. The treated animals recovered, whereas three animals that were dosed but received no therapy died between 48 and 72 hr after administration.

A case of suspected poisoning in cattle was seen after herbicide treatment of road shoulders and ditches with a spray containing simazine, aminotriazole, and dalapon (Schmoranzer et al. 1974). Fodder from adjacent meadows was given to a herd consisting of 110 young bulls, 120 cows, and 20 heifers. Indigestion and apathy were observed 4 d after spraying in 3 bulls, 1 cow, and 1 heifer. The latter delivered an 8-mon premature calf, which was described as weak but was not adequately examined. The 5 adolescent and adult cattle recovered within 5–8 d. Presumed metabolites of simazine were demonstrated chromatographically in grass samples and calf tissue; methods to test for residues of the other compounds were not available.

Another field report covered sheep that grazed weeds during or soon after spraying with simazine and aminotriazole at recommended rates (Egyed et al. 1975); 30 of 150 ewes developed a staggering gait and died within 3–7 d. The dose was estimated, based on use rate and feed intake, as 18.7 mg simazine and 6.2 mg aminotriazole per kg bw. These levels are significantly lower than established toxic doses (Hapke 1986). On separate occasions, two horses were lost within 24 hr of grazing on similar pastures.

V. Discussion

A. Chronic Exposure of Humans

According to manufacturers' experience, professional exposure to triazines under factory conditions does not affect worker health. The consistent subjective judgments of physicians caring for factory personnel were corroborated by the outcome of two epidemiology studies. A retrospective mortality study pooling the data of two U.S. cohorts (Delzell and Sathiakumar 1992; Sathiakumar et al. 1992) showed only the healthy worker effect. A slight excess of NHL did not correlate with the length and intensity of exposure and thus appears to be coincidental.

The entire morbidity spectrum was the object of a historic-prospective study on 154 Swiss matched worker pairs (Gass 1991). The clinical follow-up of a statistically significant excess of gastrointestinal complaints in the triazine cohort revealed heterogeneous pathology. Furthermore, there

was no relationship to exposure time, and no corresponding effect was recorded in the cohorts in the United States. These considerations render a causal connection between triazines and gastrointestinal effects in the Swiss cohort highly unlikely.

The absence of plausible effects of triazine exposure on the health of industrial workers deserves special emphasis because triazines have a long history of manufacture in very large amounts (IARC 1991). In the early decades of production, industrial hygiene had a lower priority if a product was considered safe, as was the case with triazines. No exposure monitoring data are at hand, but the daily exposure of a manufacturer in the 1960s and 1970s must have been much higher than a farmer's exposure on a few days each year. Because this situation caused no adverse reactions, agricultural use of the triazines can hardly lead to exposure levels entailing a hazard potential.

Several case-control and case-referent studies conducted in U.S. farming populations compared the incidence of malignant changes among agricultural users versus nonusers of triazines, especially atrazine. No association of atrazine was demonstrable with leukemia (Brown et al. 1990); slightly significant risks resulted for small-cell lymphocytic lymphoma (Cantor et al. 1985) and colon cancer (Hoar et al. 1985). Results were inconsistent for NHL. The highest OR was 2.2 in a small, heterogeneous sample of cases exposed to several triazines and other herbicides (Hoar et al. 1986). Insignificant increases of incidence in one cohort reached significance in the cohort's long-term users (Hoar Zahm et al. 1988, 1990). In another study, however, an elevated risk was mostly associated with concurrent use of other pesticides, especially in long-term users (Hoar Zahm et al. 1993). The inconsistency in the morbidity spectrum and in background conditions in these reports suggests no increase in the risk of malignant disease attributable to atrazine contact.

A significantly increased risk of primary ovarian tumors in residents of areas using herbicides was derived from data in two subsequent case-control studies in an Italian hospital (Donna et al. 1984, 1989). A statistical review suggested that the differences could result from chance (Minder 1990). As the authors assumed that work in treated fields leads to significant exposure, this contention is questionable. Maize fields are treated during a few days each year; the active ingredient is adsorbed in the soil and leaves no dislodgeable residues on the growing plants. With the exception of mixing/loading and spraying, which usually are male activities, there is therefore little chance of significant exposure, and an association of the active ingredient with female health problems is the least likely. It is unfortunate that the favorable industrial experience was gained with all-male cohorts. This leaves room for a hypothesis that triazines have a singular carcinogenic effect in the human ovary. The respective data stand alone, however, and without supporting evidence from parallel studies they do not appear plausible (Maroni and Fait 1993).

B. Acute/Subacute Poisoning in Humans

The amounts of atrazine ingested in two medically documented cases of intended suicide were enormous: 500 g and 1000 g, respectively. These amounts are equivalent to a two- to four-fold LD_{50} in rats, but attending physicians reported no signs of poisoning due to atrazine. Therapeutic measures were required to control adverse effects of coformulants that under "normal" conditions are innocuous, or to neutralize inappropriate therapy. These cases suggest a virtually unlimited tolerance of the human system to atrazine. Were it not for the intercurrent reaction to accompanying substances, they would never have been reported.

Only two cases of suspected or possible atrazine poisoning have been reported in the literature. A causal link between atrazine and the clinical observation is questionable in both cases. In the first case (Pommery et al. 1993), 100 g atrazine and 25 g aminotriazole were ingested. Much larger amounts of atrazine had been tolerated in other cases reported above; the acute toxicity of aminotriazole is even less than that of atrazine (Worthing 1991). The ingested product also contained 25 g ethylene glycol and 0.15 g formaldehyde; lethal amounts of the latter two substances are about 100 g and 10 g, respectively (Velvart 1993). Yet the patient developed signs of severe ethylene glycol poisoning and was treated accordingly. Despite therapy, he died after 3 days. The authors declared the terminal condition "not typical of ethylene glycol poisoning." This opinion is not supported by pathology findings, and diagnostic signs are described that appear compatible with fatal ethylene glycol ingestion. On the other hand, the dose that is associated with the initial poisoning, and presumably with the death, was found innocuous by other authors (Wirth and Gloxhuber 1981). Thus the case seems insufficiently investigated. The data do not support a postulated poisoning action of atrazine, which in other cases proved extremely tolerable.

Exposure data are completely lacking in the second case (Gallois and Dereux 1979). A worker with an anamnesis of diabetes and past alcoholism complained about limb weakness during a deweeding campaign. The signs were reminiscent of the clinical findings that are encountered in triazine-poisoned cattle (Section IV). For want of another etiological lead, atrazine and aminotriazole were therefore implicated. This association stands alone in 38 yr of field experience and cannot be supported.

The paucity of poisoning case reports after the long, large-scale worldwide use suggests that atrazine, like other *s*-triazines, is virtually safe for the user. Even when formulated atrazine was ingested with suicidal intent, the very large doses failed to elicit a toxic response. In two cases reported above, in which intentional heavy intake or repeated negligent exposure were associated with clinical effects, atrazine was used in combination with aminotriazole. This mixture is often used in roadside and industrial weed control. An analogous combination, with simazine rather than atrazine,

was implicated in two poisoning cases of grazing cattle. Aminotriazole has a negligible acute toxicity, whereas prolonged administration inhibits thyroid activity. Interaction of this effect with triazine toxicity is not expected. In laboratory studies in rats, acute toxicity data of commercial formulations containing simazine and aminotriazole indeed show no evidence of a potentiating effect (Ciba-Geigy 1994, personal communication). The sporadic reports of poisoning by atrazine or other *s*-triazines, alone or in combination, may be attributed to coincidence rather than to causality.

C. Acute Triazine Poisoning in Ruminants

The comparatively low tolerance of ruminants to *s*-triazines, which has been experimentally established by several authors, has led to loss of cattle that ingested concentrated products on the farm. The mechanism of this peculiar sensitivity has not been elucidated. A successful therapy is based on administering repeated large doses of activated charcoal; this method eliminates the herbicide from the enterohepatic circulation before intestinal reabsorption produces toxic tissue levels and eventual death.

A feeding study under controlled conditions (Johnson et al. 1972) showed that triazine amounts that are fatal when administered in capsules or as drench are tolerated without adverse reaction if they are adsorbed on fodder. This result contradicts reports that attribute losses of ruminants, and occasionally horses, to grazing on pasture strips exposed to triazine sprays, for example, through weed control along roadsides or drift from treated fields. The reports discussed in Section IV, and damage claims received by insurance companies, tend to base the diagnosis of triazine poisoning on the concurrence of fodder contamination with hind limb paresis and death in cattle. Yet the paretic/paralytic syndrome is unspecific. In grazing cattle, it may be caused by ingestion of yew or other toxic plants, by electrolyte imbalance, or by intestinal infection. In the absence of differential diagnosis, and provided the intake was via contaminated fodder, tolerability data (Johnson et al. 1972) present evidence against a causal link between triazine and acute poisoning signs in cattle.

Summary

Chronically exposed workers in chemical plants have revealed no increased incidence of benign or malignant disease attributable to atrazine. Some case-control studies showed a slight increase of non-Hodgkin's lymphoma (NHL) incidence while others were negative. Weighted evidence supports no causal association of malignant changes in farming populations with atrazine. Two studies on a rural population suggested an increase of ovarian tumors in exposed women. Neither statistics nor exposure data are satisfactory, however, and no other studies present supporting evidence. New studies under clearly defined conditions are desirable.

Very high doses of atrazine ingested in suicidal attempts had no acute clinical effect, suggesting that atrazine is virtually innocuous to humans. Sporadic reports on suspected acute poisoning leave too many questions open to be convincing: they reflect coincidence rather than causality.

The tolerance of ruminants to triazine is limited. Severe poisoning in case of accidental intake of concentrated products is to be expected. Poisoning through ingestion has been controlled with activated charcoal. Adsorption on fodder enhances tolerability of triazines. Suspected poisoning through spray-contaminated fodder requires differential diagnosis to avoid confusion with other pasture toxins, electrolyte problems, or gastrointestinal infection.

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Exposure of Children to Pollutants in House Dust and Indoor Air

John W. Roberts* and Philip Dickey†

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I. Introduction

Indoor pollution has been ranked by the United States Environmental Protection Agency Science Advisory Board (USEPA 1987, 1990) and the Centers for Disease Control (CDC 1991) as a high environmental risk. Together, lead, contaminated soil, house dust, allergens, volatile organic compounds (VOCs), hazardous household chemicals, indoor air pollution, tobacco smoke, and other pollutants pose a significant environmental risk to children, adults, and pets. Home pollutant exposure may result in retarded growth, learning disabilities, allergies, cancer, nervous system damage, and other illnesses. We continue to allow, in many homes, exposures to these substances that would not be tolerated on the job or in the outdoor environment because most people, including political leaders, are not aware of the problem.

Ingestion and inhalation of and contact with house dust can be primary routes of exposure for small children to pesticides, lead, and allergens (CDC 1991; Lewis et al. 1994; Platts-Mills and Chapman 1987). Most met-

*Engineering Plus Inc., 1425 E. Prospect #3, Seattle, WA 98112, U.S.A.
†Washington Toxics Coalition, 4516 University Way NE, Seattle, WA 98105, U.S.A.

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als, pesticides, and organic pollutants with relatively low vapor pressures or high polarities are expected to accumulate more in soil and dust than in air. Therefore, soil and dust can be reservoirs for pollutants from outside air or other sources. Airborne allergens are generated from molds, animals, plants, and dust mites, which thrive with the dust, moisture, skin scales, and temperature found in rugs, pillows, mattresses, and on surfaces in most houses (Platts-Mills and Chapman 1987).

Pollutants in dust increase the potential exposure of all people and pets but especially that of infants and toddlers, who crawl and mouth their hands and other objects. Dogs and cats that clean their fur with their tongue will also ingest pollutants in the house dust and soil that they contact. The average child's daily dust ingestion rate is more than twice that of adults and is estimated to be 0.02–0.20 g (Calabrese and Stanek 1991). However, 11% of toddlers may exhibit pica behavior—they eat nonfood items—and may consume as much as 10 g of soil and dust per day (Calabrese and Stanek 1991; Mahaffey and Annest 1985). Potential risks to small children relative to adults are further increased by their smaller size, higher ratio of surface area to body weight, and the stage of development of their organs, nervous system, and immune system (IPCS 1986).

Sampling house dust can give one of the best estimates of recent exposure to allergens (Pope et al. 1993) and other pollutants in the home and serve as an indication of chronic exposure to persistent pollutants. However, it has been difficult to compare one study with another, conduct epidemiological studies of exposure to house dust pollutants, or set exposure limits because of the lack of a standard method. The High Volume Small Surface Sampler (HVS3) developed for the Atmospheric Research and Exposure Assessment Laboratory of the U.S. Environmental Protection Agency (USEPA) at Research Triangle Park has been validated for measuring dust, lead (Pb), pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and allergens in carpets and bare floors (Budd et al. 1990; Chuang et al. 1994; Cole et al. 1993; Lewis et al. 1994; Roberts et al. 1993a); the American Society for Testing and Materials (ASTM) standard method D-5438-94 has been established for the HVS3. The National Cancer Institute helped develop the HVS3 for the study of childhood leukemia and of pesticide exposure of farmers. The HVS3 can collect a 2- to 100-g sample in 15 min for multiple analyses. A modification of the HVS3 labeled the HVFS (High Volume Furniture Sampler) is available for sampling pollutants in upholstery (Roberts et al. 1995).

Analytical methods have been developed by the Southwest Research Institute, Battelle-Columbus, and others for analyzing pesticides, PAHs, and PCBs in many environmental matrices, including indoor air and house dust samples (Camann 1994; Chuang et al. 1994). Progress in sampling and analysis make possible improvements in exposure analysis and the epidemiological studies discussed in the following sections.

II. Indoor Pollutants
A. Lead and House Dust

House dust in carpets, on sofas, and in air ducts is both a major source and sink of pollutants in the home (Roberts et al. 1994). The lead on surfaces and the lead loading in a carpet (μgPb/m^2) are the best predictors of a toddler's blood lead (Davies et al. 1990; Hilts 1994). Dust in carpets can also be a major source of exposure to pesticides and allergens (Lewis et al. 1994; Pope et al. 1993). We believe, but have only partially confirmed, that house dust is also a major source of childhood exposure for PAHs, PCBs, and other persistent pollutants.

It has been estimated that 1 in 11 preschool children have levels of lead in their blood that exceed the definition of lead poisoning associated with reduced intelligence, growth, and hearing (CDC 1991, 1994). The portion of lead in house dust that comes from autos has been greatly reduced. However, house dust is still the major route of lead exposure for most children. Dilapidated housing, peeling paint, paint removal, and remodeling of old houses while toddlers and pregnant women are present continue to present high risks (CDC 1991). The USEPA cleanup standard for lead at Superfund sites is 500 ppm Pb in soil. The mean soil lead concentration near the foundations in 37 older Seattle homes exceeded this level by fourfold and the house dust exceeded it by twofold (Roberts et al. 1991b).

Road and street dusts are sources of lead, cadmium, PAHs, pesticides, fuel additives, and many toxic pollutants that find their way into the home. PAHs, lead, and pesticides are often found in higher concentrations in house dust than in outside soil, road dust, or bottom sediments in rivers and bays (Camann 1994; Lewis et al. 1994; Menzie et al. 1992; Roberts et al. 1992; Whitmore et al. 1994). Campbell (1937) found that 70% of mice exposed to road dust on their cage floor and in the air developed skin cancer during their lifetime; 74% developed lung cancer. When the study was repeated and the toxic organics including PAHs were removed from road dust, the skin cancer rate dropped to zero and the lung cancer rate dropped to 45%.

The amount of dust in each square meter of an older carpet may be 400 times that found on a bare floor in the same house (Roberts et al. 1987, 1994). Old rugs and sofas tend to have more dust, lead, pesticides, and mutagenic activity than newer carpets and sofas (Camann and Buckley 1994; Clark et al. 1991; Lewis et al. 1994; Roberts et al. 1994). Normal cleaning is not effective in reducing lead and pesticide exposure from rugs after remodeling an old home, if the home is near a lead-contaminated Superfund site, or after use of pesticides (CH2M Hill 1991; Roberts et al. 1991b; Whitmore et al. 1994).

The surface dust and lead removed from an old rug with normal vacuuming may be only 5%–10% of the total dust and lead that are found deep in the carpet, pad, and under the pad (CH2M Hill 1991; Roberts et al. 1994).

Deep dust tends to accumulate in carpets and to have the same concentration of lead as the surface dust. The increase in pollutants in carpets can be slowed by a factor of 10–100 and may be reduced by the cleaning methods listed in Table 1. Such control measures reduced the surface lead loading in three test rugs by an average factor of 68 over the course of a year (Roberts et al. 1991b, 1994). The cleaning methods listed in Table 1 have been tested and found to reduce children's blood lead and exposure to airborne allergens, particles, and VOCs (Charney et al. 1983; Cole et al. 1994; Mielke et al. 1992; Roberts et al. 1991b). Use of efficient vacuums, hot water extraction cleaning of rugs, good quality door mats, damp dust cloths, and dust control on hard surfaces reduced VOCs and particles in indoor air by 50%. Airborne levels of biopollutants were reduced 18–94% (Cole et al. 1994). Use of less toxic cleaning products and other home chemicals can also reduce home hazards (Dickey 1994).

B. Allergens

Allergies and asthma are major illnesses that are increasing in the U.S. and many other parts of the world. Allergic disease runs in families. The likelihood of allergic disease for children is 60%–100%, 31%–58%, or 0%–19%, respectively, if both parents, one parent, or no parent has allergic disease (Pope et al. 1993). Forty percent of the U.S. population has become sensitized to allergens (Pope et al. 1993). Ozone, NO_2, SO_2, VOCs, and particulate air pollution have been linked to sensitization and asthma (Koenig et al 1993; Pope et al. 1993). Asthma prevalence in the U.S. was 0.5% in 1930, 4%–6.7% in the 1970s, and 8%–12% in 1991 (Pope et al. 1993). A gradual increase in asthma incidence began in the 1940s and has continued to the present (Pope et al. 1993). The U.S. asthma hospitalization rate of children and older people, who spend the most time in the home, doubled from 1978 to 1982, with the rate for children increasing from 14 to 28 per

Table 1. Effective cleaning.

1. Reduce track-in by shoe removal and/or use of a high-quality door mat.
2. Use an efficient vacuum cleaner with a power head once a week on rugs and floors (twice a week in homes with a crawling child).
3. Once a month, vacuum and/or wet-wash surfaces that may be touched (furniture, windowsills, children's toys, and car interiors). Hand vacuum with a power head on plush upholstery.
4. Choose furniture, floor coverings, and curtains that are easy to clean. Bare floors, flat rugs, and flat upholstery are easier to clean than plush rugs or upholstery (Roberts 1991b).
5. Clean carpets annually with a truck-mounted hot water extraction system.
6. Clean air ducts annually.
7. Use the least-toxic products available for cleaning and other home uses.

10,000; the rate reached 35/10,000 in 1992 (NHLBI 1994a). The hospitalization rate for children aged less than 15 yr continues to increase even though other age groups have leveled off or started a downward trend. The increased moisture levels and poor air quality found in homes and offices that were tightened to reduce energy use in 1974 following the energy crisis may be related to the rapid increase in asthma rates that took place from 1978 to 1982 (Robinson and Russell 1992).

Childhood asthma hospitalizations in Seattle rose from 306 in 1988 to 612 in 1993, an average increase of 14.4%/yr for children under 15 yr of age (WSDH 1994), compared with a 4%/yr increase in the U.S. as a whole. Asthma-related hospital costs in Seattle rose more than 300% in these same 6 years from $614,000 to $2,526,000. Seattle hospital rates were 56/10,000 in 1993 (Fig. 1) compared with 35/10,000 per year for the U.S. as a whole (NHLBI 1994a; WSDH 1994). These rates were based on the 1990 census data, adjusted by using the Seattle public school (unpublished) population for October of each year. The average 1993 rates (90.8) in the four Seattle zip code areas with the most Medicaid cases averaged five times that (17.9) found in Redmond, a suburb of Seattle (WSDH 1994). Asthma is the leading cause of hospitalization of preschool children in Seattle and the leading cause of school absence in the U.S. (Batik et al. 1992; Pope et al. 1993).

Asthma in the U.S. was estimated to cost $6.2 billion in 1990 (Weiss et al. 1992a,b). However, asthma-related visits to hospitals and school absence will be rare if the National Heart, Lung and Blood Institute (NHLBI 1994b) Guidelines for the Diagnosis and Management of Asthma are fol-

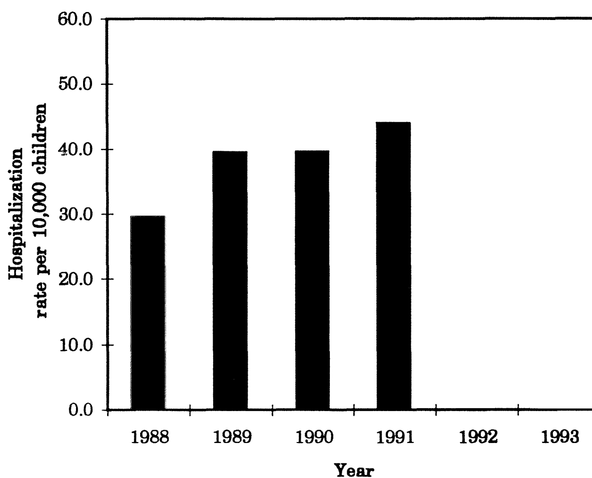

Fig. 1. Annual Seattle childhood asthma hospitalization rate.

lowed. These guidelines provide for diagnosis, patient education, self-monitoring, medication, and control of environmental exposures. The costs and severity of asthma and allergy, as well as the risk of being sensitized and developing allergic disease, can be greatly reduced by reducing exposure to indoor allergens (Pope et al. 1993).

Dust mite exposure in indoor air and dust in the home appears to be the main source of allergies and asthma. An estimated 8%–12% of the general population suffers from dust mite allergies (Platts-Mills et al. 1987); 16%–20% have rhinitis; and 11%–13% have sinusitis (Pope et al. 1993). As many as 80% of people can become sensitized to dust mites with high cumulative exposure (Pope et al. 1993). Infants who are exposed to wood smoke, tobacco smoke, and dust mites tend to have more asthma and lesser lung function than other children (Koenig et al. 1993; Sporik et al. 1990). Mite feces and their dead body parts readily become airborne from activity in the room or movement in bed. Cities with mild and damp weather such as Seattle will tend to have higher than average dust mite populations, with related higher rates of allergies and asthma. Mites thrive deep in old carpets, mattresses, quilts, and sofas. Sofa dust collected from ten used couches in Seattle averaged 16.3 μg/g for mite allergen and 37.2 μg/g for cat allergen (Roberts et al. 1995). The concentration of dust mite allergen on mattresses, sofas, or carpets that is associated with triggering an asthma attack has been established at 2 μg/g (Ehnert et al. 1992). The use of mite-proof mattress and pillow covers, washing of bedding in hot water, removal of carpets and fleecy material from the bedroom, and humidity control are effective in reducing allergies and ashtma related to dust mites (Ehnert et al. 1992; Murray and Ferguson 1983). Such control measures are also associated with reduced costs, hospitalizations, medicine, and medical treatment.

Army trainees housed in modern, energy-efficient, and "tight" barracks built in the late 1970s or early 1980s had a 50% increase in fever-related acute lung disease when compared with trainees housed in older barracks with more frequent air exchanges (Brundage et al. 1988). One in three homes has high relative humidity that favors dust mites, molds, asthma, allergies, and other respiratory problems (Brunekreef et al. 1989; Tsongas 1992). Excess moisture in buildings is also associated with large maintenance costs from damage to window frames, walls, floors, and wood framing.

The sick building syndrome (SBS), characterized by upper respiratory complaints, headache, rashes, fatigue, watery eyes, inability to concentrate, dizziness, and other complaints by building occupants, is often found in energy-efficient buildings (Robinson and Russell 1992). Most of the factors associated with the SBS (poor ventilation, dust, dusty rugs, other fleecy surfaces, moisture, molds, and VOCs) in office buildings are also found in the home (Wallace 1987). Remodeling a home or office, which can increase dust, lead and toxicants in dust, and VOCs in air, has been associated with

SBS and lead poisoning (CDC 1991; USEPA 1993b). The Danish Town Hall Study (Skov et al. 1989, 1990) found a significant relationship between SBS and measured dust, the organic fraction of the dust, and two factors that may be correlates of increased dust: the "shelf factor" (amount of shelf space) and "fleece factor" (amount of fabric-covered space). A later study of 12 Danish town halls and 870 individuals showed a correlation between the qualitative properties of dust and the SBS. The following correlations were observed: gram-negative bacteria with fatigue, heavy-headedness, headache, dizziness, concentration problems, and irritation of the mucous membranes of the upper respiratory tract; particles in dust with symptoms from the mucous membranes; total VOC in dust with lack of concentration and heavy-headedness; potentially allergenic material with headache, general malaise, and dizziness; and the ability of dust to stimulate the liberation of histamine with general malaise, dizziness, and lack of concentration (Gyntelberg et al. 1994).

Adults raised in West Germany after 1945, when Germany was divided, have higher levels of allergy and sensitization to allergens than adults raised in East Germany after 1945. For men aged 24–30 yr, 17.2% in West Germany and 10.3% in East Germany reported allergies. For the same group, 29.8% in West Germany and 20.3% in East Germany showed sensitization to allergens. The levels of soot and SO_2 in most areas of East Germany were much higher than in West Germany, but the levels of auto-related pollution (NO_2 and ozone) were higher in West Germany. Higher levels of sensitization were found in larger cities and with higher levels of education in both East and West Germany. The observed difference of allergies widens with lower age. These findings strongly suggest that the main causes of sensitization are found in early childhood exposures (Bellach et al. 1994). Also, higher levels of ozone may increase the impact of dust mites. Plans are being made in Germany to evaluate hypotheses related to preschool exposures, genetic factors, and immune system effects to explain these differences.

C. Pesticides

Pesticide use around the home provides a significant potential for human exposure in house dust and indoor air. The California Department of Food and Agriculture reported that a sizable number of persons in California required medical attention or contacted a poison control center after use of pesticides such as flea bombs and lawn sprays (Knaak et al. 1987).

Pentachlorophenol (PCP) is widespread in house dust in the U.S. and has been found in German house dust in homes where wood preservatives had been used (Krause et al. 1987; Lewis et al. 1994). Dust concentrations of PCP were observed to be correlated to air concentrations in homes in which the application had occurred within the past 2 years, but the correlation was absent if the application had been performed more than 2 years

previously. The concentrations of PCP in dust tended to remain relatively high, while the concentrations in air dropped by a factor of about 4 after 2 years.

The air and dust concentrations for the five most frequently detected pesticides in 18 homes showed significant correlations ranging from $r = .63$ to $r = .88$ (Camann et al. 1991, 1993). However, in a Florida study 13 pesticides were found in dust in nine homes when these same pesticides could not be detected in the air (Budd et al. 1990). These pesticides and PAHs in rug dust may result from carry-home from a job or other sources away from the home. Carpet dust may, therefore, be a broader indicator of a child's exposure for some pesticides, PAHs, and other toxicants than indoor air, outdoor air, or soil samples.

D. Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls

PAHs, PCBs, and dioxins are widely spread in air and soil (Travis and Hester 1991) and have been listed as probable human carcinogens (IARC 1983). The sum of the seven potentially carcinogenic (B-2) PAHs in eight houses in Columbus, Ohio averaged 60.3 μg/g and ranged from 11 to 290 μg/g (Chuang et al. 1995; Roberts et al. 1993a). Benzo(a)pyrene (BaP) ranged from 1.5 to 44 μg/g. The sum of the same PAHs in nine houses in Seattle averaged 5.3 μg/g and ranged from 3 to 12 μg/g; BaP ranged from 0.73 to 1.7 μg/g (Chuang et al. 1995). There were no obvious sources of these PAHs (such as traffic, cooking, and smoking), with the exception of the highest concentration in Columbus, where the driveway had been paved with asphalt. Cooking can be a major source of PAHs that have four rings or less (Venkataraman and Friedlander 1994). However, the most toxic B-2 PAHs, such as BaP, have more than four rings. The total PCBs in house dust in these same houses in Seattle and Columbus ranged from 264 to 804 ng/g and 210–1900 ng/g, respectively (Chuang et al. 1994, 1995).

Camann and Buckley (1994) used the HVS3 to collect house dust from the homes of more than 250 children with acute lymphocytic leukemia and 250 control children in nine states: Illinois, Indiana, Iowa, Michigan, Minnesota, New Jersey, Ohio, Pennsylvania, and Wisconsin. A preliminary analysis of 28 pesticides, 10 PAHs, and cotinine has been compiled on 362 cases and controls. The most frequently found pesticides, the percentage of homes where found, and median values, respectively, were as follows: *ortho*-phenylphenol, 96%, 0.48 μg/g; chlorpyrifos (Dursban®), 67%, 0.46 μg/g; carbaryl, 32%, 0.54 μg/g; DDT, 25%, 0.25 μg/g; chlordane, 16%, 0.86 μg/g; and dieldrin, 13%, 0.25 μg/g.

Benzo(a)pyrene was found in 89% of the homes at a median value of 1.12 μg/g. The sum of the medians of the seven B-2 PAHs in house dust was 7.22 μg/g. These B-2 PAHs show strong within-home covariance and were detected in 90% of the homes. Cotinine, which comes from tobacco smoke, was found in the rug dust in 77% of the homes at a median level of

2.12 $\mu g/g$ even though only 28% of Americans smoke. These data are valuable in that the controls were selected at random. They tend to confirm the PAH and pesticide house dust data collected in earlier nine home studies in Florida, Ohio, North Carolina, and Washington (Budd et al. 1990; Chuang et al. 1994, 1995; Lewis et al. 1994). However, these data from Camann and Buckley are considered preliminary and subject to revision in that the controls have not been separated from cases and the analysis and quality control process has not been completed on the more than 500 cases and controls that have been collected.

Many PAHs and other pollutants in house dust may also cause gene mutations. In a study of 29 Seattle homes, house dust samples were tested for genetic toxicity using a DNA repair assay (Warren 1982) and the Ames test (Maron and Ames 1983) for mutagenic activity (Roberts et al. 1987). Significant scores on the Ames test, greater than double background rates, were found in 12 samples; 20 samples showed the ability to interfere significantly with DNA repair. A positive correlation was observed between mutagenic activity of the house dust and age of the carpet. However, the major sources of PAHs and the mutagenic activity in house dust are unknown. Because of the high correlation between PAHs in the door mat and the carpet, it appeared that track-in was the major pathway for rug PAHs in the Columbus, Ohio study (Chuang et al. 1995).

E. Indoor Air Pollutants

Radon in indoor air may cause 14,000 cancer cases and secondary tobacco smoke may cause 3,000 cancer cases each year (USEPA 1993b). USEPA has developed effective methods to monitor and manage radon. They recommend that every home purchase a low-cost monitor to assess exposure and determine what control steps if any are needed. The risk from radon is increased by the presence of secondary tobacco smoke, which contains many toxic compounds such as PAHs and benzene (USEPA 1993b; Wallace 1991). Thanks to public education about risks from radon and tobacco in the home, many families will no longer accept smoking inside the home.

The upper bound of cancer risk from exposure to pesticides, VOCs, and PAHs (including secondary tobacco smoke) in indoor air may exceed one in 1,000 (Wallace 1991). When radon is included, the risk of cancer may be greater than 1 in 100 in many homes. VOCs, biopollutants, and dust (both perceived and measured) have been associated with SBS in office buildings (Raw et al. 1993; Roberts et al. 1992).

III. Comparison of Home and Other Exposures and Risks

The USEPA sets Superfund soil cleanup standards for PAHs on the basis of a site-specific risk assessment. The Seattle Harbor Island PAH cleanup standard for soil at an industrial site was set at 22.6 $\mu g/g$ for total hazard-

ous PAHs, which include the B-2 PAHs (Mclaren-Hart 1994). The Washington State Model Toxics Control Act (MTCA) sets cleanup standards of 250 ppm (μg/g) for lead, 1 ppm for the sum of the potentially carcinogenic (B-2) PAHs, and 1 ppm for PCB mixtures for residential soils at hazardous waste sites (WDOE 1993). USEPA (1993a) set a residential soil cleanup level of 0.172 μg/g for carcinogenic PAHs at the American Crossarm and Conduit Superfund site in Chehalis, Washington because of the presence of two other carcinogenic compounds, pentachlorophenol (PCP) and dioxins. The cleanup level for PCP and dioxin was set at 8.33 μg/g and 0.0066 ng/kg, respectively, (USEPA 1993a): 12–290 μg/g of these same PAH compounds were found in rug dust in Columbus, Ohio; 3–12 ppm in Seattle; and a median concentration of 7.22 μg/g in 362 homes in nine states (Camann and Buckley 1994; Chuang et al. 1994, 1995). The MTCA does not apply to house dust or foundation soil. However, the concentrations of lead, PCBs, and PAHs measured in house dust significantly exceed the MTCA cleanup standards for residential soils in many of the sampled houses in Seattle, Columbus, and elsewhere in nine states.

The total number of cancer cases and other severe health effects associated with exposure to indoor air and dust in the U.S. is estimated in Table 2.

The information in Table 2 compares with 9,100 deaths and 3,000,000 injuries on the job and 38,100 deaths and 1,800,000 injuries from autos in 1993 (National Safety Council 1994). It has been estimated that 20 toxic air pollutants caused 2,000 cancers annually (USEPA 1987). Superfund and hazardous waste sites were projected to cause 1,000 and 100 cancer cases, respectively, per year (USEPA 1987). The health damage from pollutants in the home is in the same range as deaths and injuries caused by cars and on-the-job accidents. The number of cancer cases listed for home toxics is far in excess of the estimated 5–27 cases/yr used by USEPA to regulate

Table 2. Estimated number of cancer cases and other severe health effects associated with exposure to indoor air and dust (U.S.).

Estimated cancer cases per year:	
Indoor radon (USEPA 1993b)	14,000
Secondary tobacco smoke (USEPA 1993b)	3,000
Indoor 12 VOCs and 23 pesticides (Wallace 1991)	3,000
Cases from carcinogens in house dust not estimated	?
Deaths from asthma (Pope et al. 1993)	4,000
Other health effects (total number of people affected)	
Lead damage to children (CDC 1994)	1,500,000
Asthma (8%–12%) (Pope et al. 1993)	25,000,000
Allergic rhinitis (16%–20%) (Pope et al. 1993)	47,000,000

hazardous outdoor air pollutants (Wallace 1987) or the 48 cancer cases during 45 yr used by the Occupational Safety and Health Administration (OSHA) to regulate formaldehyde exposures on the job (OSHA 1987).

IV. Discussion and Conclusions

Infants and toddlers receive a broad and significant range of exposures to lead, pesticides, PAHs, toxic home chemicals, and allergens. These home exposures routinely exceed those tolerated on the job, in the ambient air, or at a Superfund site, and the 11% of toddlers who have pica may receive the highest exposure. Current regulations of exposure to pollutants in outside air, water, and food do not adequately address the risks in the home from pollutants in soil, house dust, indoor air, moisture, and home chemicals. Reducing residual toxic emissions to the air as required by the Clean Air Act of 1990 needs to be complemented with a plan for reducing exposures from the residuals that remain in soil, house dust, and indoor air that may be related to past emissions and track-in as well as present sources in the home. Reducing these exposures can reduce risks for allergy, asthma, respiratory disease, lead poisoning, and cancer.

Davis et al. (1994) noted a twofold increase in cancer rates among nonsmoking White males born in the 1940s compared with nonsmoking White males born in 1888 through 1897. These changes were not linked to aging of the population. In light of these findings and similar results in Sweden as reported by Davis et al. (1994), it appears that environmental exposures to carcinogenic compounds, including those that occur in the home, should be studied further as a likely source of such changes in cancer rates.

The acceptable risk of cancer from exposure to ambient air pollutants is usually considered to be 1/1,000,000 persons. The risk of cancer at Superfund sites is usually limited to 1/10,000. However, the risk of cancer from pollutants in air and dust in the home may exceed 1/1,000. The lead risks from soil at Superfund sites is set (500 ppm Pb) so that fewer than 1 in 20 children will have lead poisoning, but the average lead concentration in house dust in older homes may exceed 1000 ppm, and 1 toddler in 11 has lead poisoning. At least 1 person in 5 has allergies and asthma related to exposures to allergens in the home.

Pesticides, PAHs, lead, and PCBs are frequently found at higher concentrations in house dust than in soil near the foundation or on home walkways. It appears that the small particles, with their higher concentrations of pollutants, are more likely to stick to shoes and be tracked in. Organic pollutants, which are broken down by sunlight, moisture, and soil bacteria may be magnified more than metals in house dust. Metals do not degrade in either soil or house dust, whereas organics are protected from degradation.

Effective methods are available for control of house dust and indoor air pollutants. These include removal of shoes at the entry way, use of commer-

cial door mats, frequent use of efficient vacuum cleaners and other types of cleaning, and reducing the use of toxic chemicals. Such methods have been tested and found to reduce children's blood lead and exposure to airborne allergens, particles, and VOCs. Avoiding the use of mothballs and air fresheners with *p*-dichlorobenzene, for example, reduces the risk of cancer. Avoiding storage of gasoline and lawn mowers in attached garages means less risk of fire and of cancer from benzene.

While we have learned much about sampling and exposures in house dust, additional research is needed to understand and manage such exposures. Existing studies already provide an adequate basis for setting public policy for pollution prevention in the home. The National Human Exposure Assessment Survey (NHEXAS) studies planned by the U.S. EPA will supply much-needed data on exposures in the U.S. Preliminary risk assessments based on present data suggest giving high priority to public education. There is also a need for studies to determine the source of PAHs and mutagens in house dust.

The rapid increase in asthma hospitalizations of children in the U.S. as a whole and in Seattle can be reversed if the NHLBI guidelines for asthma are followed. A health maintenance organization (HMO) in Hawthorne, California reduced asthma hospitalization rates from 66 to 22 per 10,000 and the associated costs by 74% by having an allergist train their pediatricians (Jancin 1995), but one survey found that only 25% of the primary care physicians had adopted the NHLBI procedures for treating asthma (Jancin 1995).

The effectiveness of measures to control allergies and asthma in reducing health costs appears easier to test than measures to reduce the incidence of cancer, which has a much longer latent period. The financial impact of reducing lead poisoning (CDC 1991) is very large but not as immediate as the benefits from control of allergies and asthma. There can be an immediate benefit for children with dust mite or cat allergies or asthma if the exposure to these triggers is reduced below the threshold level. The costs of the reduced medicine, doctor and emergency room visits, and hospitalizations are relatively easy to determine. It may be possible to channel these savings into prevention programs. The expenditure of $10 to $45 for mite-proof mattress and pillow covers lowers the risk of a visit to the hospital, estimated to cost $5500 in Seattle for a child on Medicaid (WSDH 1994). Once these covers are installed, they require very little maintenance. Such covers are FDA registered medical devices.

The Master Home Environmentalist Program (MHEP) is an example of a public education program designed to reduce exposures by one-on-one contact in the home. The MHEP is funded by the U.S. EPA, Region 10, METRO, Seattle-King County Department of Public Health, and the Washington State Department of Ecology. Some 100 volunteers have each received 40 hr of training as of May 1995 and are now doing free home assessments and follow-ups to reduce exposure to indoor air pollutants,

house dust, home chemicals, and soil pollutants (Roberts et al. 1993b). The MHEP may be one of the fastest ways to transfer effective cleaning and control methods to motivated families. Home vacuums have been used by the MHEP as a low-cost way to measure dust and lead exposure in carpets and to provide reinforcement for effective cleaning. Commercial Kex and Coral door mats distributed in public education programs by the League of Women Voters and the Master Home Environmentalists in Seattle have been very popular for controlling track-in.

Those studies conducted to date establish a basis for control of lead, pesticides, PAHs, PCBs, and allergens in indoor air and house dust. Track-in and accumulation of pollutants in rugs is an important source of exposure for children; lead in dust is the major source of lead for most toddlers. The established benefits from reduced exposure to lead and allergens justify public education to reduce dust from track-in and inside sources as well as pollutants in indoor air. Action to control lead and allergens will reduce exposure to other pollutants in house dust and indoor air. It is possible that a nonregulatory education and outreach program with emphasis on public participation and pollution prevention will be more effective in the home than use of regulations and other traditional methods of pollution control.

Available evidence shows that frequent, thorough cleaning can reduce exposure to a range of home pollutants. Commercial buildings use efficient door mats, vacuums, and cleaning methods to lower cleaning costs and to attract customers. Cleanliness also makes a home more attractive. However, more parents have fulltime jobs outside the home now than 20 years ago and have less time for cleaning; many place a lower priority on cleaning relative to the other problems they face. All of these factors increase the need for effective cleaning training to reduce time and costs.

The 20% of the population with allergy or asthma have added motivation to make changes in cleaning, buying, and control practices. An additional 20% who have been sensitized or who have parents with allergic disease would have additional reasons to reduce exposure if they were aware that they have a high risk of allergy or asthma. Skin tests that provide this awareness can be part of preventive medicine.

The quality of life for sufferers of asthma or allergy can be dramatically improved when they gain access to proper medical care and learn self-monitoring as well as how to control exposure to allergens. The benefits may include fewer days lost from school or work; less medication; fewer visits to the doctor, hospital, and emergency room; fewer restrictions on school activities, job placement, and recreation; less chance of losing a job; higher levels of energy and performance; and lower medical premiums (Pope et al. 1993). These quality of life improvements are an important part of school and welfare reform that can aid the schooling of children and increase their ability to work. Seattle and the rest of the U.S. face large increases in asthma and allergy costs if the present trends continue. Proven

methods of intervention that reduce costs will become more and more attractive.

The 4,000 asthma deaths and 20,000 cancer cases estimated from home-related exposures are eightfold larger than the 3,000 cancer cases estimated for 20 toxic air pollutants and all Superfund sites. The 50,000,000 injuries/yr in the U.S. from lead and allergic disease in the home can only be compared with the total health damage from ambient air and water pollution. However, it will be difficult to establish or change environmental and personal priorities on the basis of these opportunities for risk reduction if the public and our leaders are unaware of them.

More research is needed to determine the cause of the rapid increase in childhood asthma in Seattle and the U.S., as well as the source of PAHs and mutagens in house dust. There is a need to use existing data to assess risks and set priorities for risk reduction. Perhaps the highest priority should be given to finding cost-effective methods of public education to reduce home exposures. Such methods may vary with the level of income and education. We recommend the following:

1. Estimate risks for lead, pesticides, PAHs, VOCs, and PCBs in house dust and indoor air using available data. These risk assessments should include synergistic interactions between pollutants that may increase total risk.
2. Determine the source of PAHs and mutagens in house dust. Determine the concentration of dioxins in house dust.
3. Determine the factors affecting the exposure of infants and toddlers to pesticides and PAHs as well as the relationship between dust exposure and biomarkers.
4. Determine if the Master Home Environmentalist Program can reduce asthma costs in a controlled study in Seattle.
5. Do research on other public education methods for reducing home exposures for low- and middle-income groups.
6. Build a model to show the factors that affect reducing or eliminating the accumulation of dust and pollutants in wall-to-wall and area rugs.
7. Determine the cause of the increase in asthma hospitalization rates of children in Seattle and the U.S.
8. Incorporate public education about ventilation and control of indoor pollutants in energy conservation programs.
9. Determine the costs and benefits of improving ventilation and moisture control in existing housing.

Summary

This review summarizes occurrence and exposure studies for pollutants in house dust and related indoor air exposures. A standard sampling method and control methods to reduce these exposures are discussed, including recommendations for future research.

Infants and toddlers receive a broad and significant range of exposures to lead, pesticides, PAHs, allergens, and VOCs in house dust and indoor air. Carpet dust in eight Columbus and nine Seattle homes contained concentrations of potentially carcinogenic PAHs ranging from 3 to 290 μg/g, of lead from 250 to 2250 μg/g, and of PCBs from 210 to 1900 ng/g. Dust collected from ten used sofas in Seattle averaged 16.3, 37.2, and 229 μg/g for dust mite allergen, cat allergen, and lead, respectively; dust samples showed mutagenic activity. Biological and chemical pollutants in indoor dust and air have been associated with lead poisoning, cancer, allergy, asthma, damage to the nervous system, and sick building symptoms. The 11% of toddlers who have pica tend to have the highest exposures and risks. Further, the exposure of toddlers to lead via the dust pathway can be greater than by other routes. Standard method ASTM 5438-94 for sampling house dust has been used to characterize current and chronic exposure of toddlers in epidemiological studies. The accumulation of dust, dust mites, and tracked-in soil in old carpets, sofas, and mattresses appears to be a major source of exposure to lead, pesticides, allergens, PAHs, and VOCs. Remodeling and energy conservation can reduce ventilation and increase relative humidity, dust, dust mites, molds, VOCs, and other indoor air pollutants.

The U.S. faces large and increasing costs from asthma and allergy. Asthma incidence in the U.S. has increased from 0.5% in 1930 to 8%–12% in 1991. Asthma hospitalization rates for children are increasing at the rate of 4%/yr in the U.S. and 14%/yr in Seattle. Such hospital visits would be rare with effective diagnosis, patient education, and control of home exposures. Asthma was estimated to cost $6.2 billion in 1990; hospital visits of children in Seattle cost $2,526,000 in 1993. Forty percent of the U.S. population has been sensitized to allergens; one in three homes has high relative humidity, which favors dust mites, molds, allergies, asthma, and other respiratory diseases. Reducing indoor allergens can reduce costs, severity, and the risk of being sensitized and developing allergic disease. Use of volunteer Master Home Environmentalists to do free in-home surveys and education in Seattle may reduce immediate health costs from allergens as well as long-term risks from lead, carcinogens, and home chemicals. Public education can lead to more effective cleaning and pollution prevention: use of efficient vacuums and quality door mats, removing shoes at the door, ventilation, and allergen control; most of these measures are low cost. Home pollution prevention programs need to be tested for low- and middle-income groups. Families should be made aware of home risks and the need for control.

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Bioaccumulation of Polycyclic Aromatic Hydrocarbons by Marine Organisms

J.P. Meador,* J.E. Stein,* W.L. Reichert,* and U. Varanasi*

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I. Introduction

Polycyclic aromatic hydrocarbons (PAHs) appear in most urbanized coastal areas of the world, accumulating in sediments and biota that are unable to efficiently eliminate them. This review focuses specifically on the mechanisms of bioavailability, uptake, and elimination, which determine the extent of accumulation and retention of PAHs in invertebrates and fish in marine ecosystems. We review here the literature on the mechanisms and factors that control these processes which ultimately determine the concentration of PAHs in marine organisms. Understanding both the tem-

*Environmental Conservation Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, WA 98112, U.S.A.

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poral and spatial characteristics of bioaccumulation of the environmentally important PAHs is crucial for determining the impact that this class of compounds may have on marine populations. To provide a complete assessment of these potential impacts, scientists require knowledge about the distribution of these compounds in different environmental matrices, their uptake and partitioning in different tissues, their rates of elimination, and their potential for persistence in certain species. The combined information on these mechanisms and the environmental factors that control accumulation will help scientists develop predictive models of contaminant accumulation both for acute events, such as oil spills, and for long-term, chronic exposure as is found in many urban areas in our coastal ecosystems.

Bioavailability and organism physiology are the two important variables that have a major effect on chemical contaminant body burden. Of the total environmental concentration, only the bioavailable fraction can enter the organism. Unlike that of metals and ionizable organics, the bioavailability of PAHs is affected by only a few environmental variables such as organic carbon and sediment surface area. Physiological factors, including lipid levels and the rates of uptake and elimination (metabolism, diffusion, and excretion), also determine contaminant body burden.

Uptake is an important determinant of body burden that is controlled by factors associated with bioavailability and organismal physiology. It is believed that the process of uptake of hydrophobic compounds is passive (vis-á-vis active transport) and controlled by diffusion pressure (fugacity) because of the differential between the environmental matrix and tissue concentration. For uptake of PAHs from water, factors that control the concentration of the free PAH (nonsorbed) are important. Variables such as salinity and hydrogen ion concentration may also have an effect on the bioavailable fraction in water; however, these factors generally have a relatively minor effect over the range found in marine and estuarine environments. Ingestion of prey organisms, detritus, and sediment is also important for PAH accumulation; however, the factors that determine the bioavailable fraction taken up from ingested materials are less well known. It has been demonstrated that the rates (Bender et al. 1988; Landrum 1988) and efficiencies (McKim et al. 1985) of uptake vary slightly among PAHs and are therefore not strongly linked to chemical hydrophobicity. It may be that the rate-limiting step for uptake is at the water–membrane interface, where the ordered structure of water is broken.

The elimination of PAHs can occur by biotransformation of parent compounds, diffusion when the external concentration favors outward flux, and excretion of parent or metabolite compounds. This review presents studies that show a differential ability of species and tissues to biotransform PAHs to more water-soluble metabolites, which results in differences in body burden and half-life. Some studies indicate that a persistent PAH fraction exists in the tissues of organisms that are exposed for long periods to PAHs, even in species capable of metabolizing these xenobiotics.

Studies on PAHs are largely conducted with specific compounds. For example, many studies that explore the uptake and elimination of PAHs in marine organisms deal with naphthalenes, a major component of petroleum, and carcinogenic PAHs, such as benzo[a]pyrene. Petroleum spills are important acute events; hence, it is important to understand the processes that control the bioaccumulation of naphthalene and related compounds. Understanding the mechanics of chronic exposure to PAHs from urban areas is also desirable when assessing the population structure of marine organisms and the possibility of contaminant transfer to humans. Additionally, the carcinogenicity of PAHs is an important concern because some PAHs are biotransformed to reactive metabolites that can interact with DNA.

The ability to predict tissue concentrations in feral organisms is important in the assessment of possible toxic effects. For example, the lethal body burden for nonionic hydrophobic compounds such as parent PAH compounds, acting by narcosis, is believed to be in the range of 2–6 μmol/g (wet wt) (McCarty 1991). For species with a weak or nonexistent ability to metabolize PAHs, assessing body burden concentrations in light of this critical body burden may be a useful first approximation of potential deleterious effects. Moreover, these body burdens could be used to characterize relationships between chemical exposure and ecological effects in marine species. In species with weak metabolic capacity for these contaminants, toxic response assessment based on actual or expected tissue concentration instead of environmental concentration (i.e., LD_{50} vs LC_{50}) would be less ambiguous because factors affecting bioavailability are eliminated. However, the ability to predict tissue burdens based on environmental concentrations has proved to be less than satisfactory for many species. While accumulations of organic contaminants in tissues are often less complicated to predict than for metals, predicting the accumulation of metabolizable compounds, such as PAHs, requires knowledge about the biotransformation capabilities of the organism.

This review provides insight on several important processes that control bioaccumulation of PAHs in marine organisms. Some of the more important points covered concern the role of organic carbon and lipid in the control of PAH partitioning, the observed anomalous sediment–water partitioning behavior, the routes by which PAHs are taken up, the rates of uptake and elimination, the role of biotransformation in bioaccumulation, the variation of bioaccumulation over a range of hydrophobic PAHs, the temporal nature of exposure and its impact on persistence, the time to reach steady-state tissue burdens, the efficiency of uptake, and the relationships between environmental concentrations and tissue accumulations. An examination of the literature related to these points will help explain the complexities and subtleties of bioaccumulation of PAHs and other organic contaminants. The abbreviations and formulas that are used here are defined in the Appendix.

II. Important Properties of PAHs in Environmental Matrices

Polycyclic aromatic hydrocarbons are defined as compounds with two or more fused benzene rings and often contain alkyl side groups. Low-molecular-weight PAHs (LPAHs) are defined here as those possessing two or three aromatic rings, and high-molecular-weight PAHs (HPAHs) as those with four or more rings. The designation of low and high molecular weight is somewhat arbitrary because the actual difference in molecular weight between 2- and 6-ring compounds is not dramatic (128.2 daltons for naphthalene to indeno[1,2,3-c,d]pyrene at 276.3 daltons) (Table 1). The major difference between PAHs is solubility in water, which can be expressed in terms of hydrophobicity. In general, PAHs are more hydrophobic as molecular weight increases. As hydrophobicity increases, thermodynamic equilibrium tends to favor partitioning of the PAH molecule to more nonpolar environments, such as the lipid of an organism or the organic carbon surrounding a sediment particle. (Equilibrium as used here is a dynamic chemical equilibrium in which the PAH interacts with organism lipid or organic carbon and inflow equals outflow.) One way to express hydrophobicity is with the octanol–water partition coefficient (K_{ow}), which can be empirically determined by laboratory experiment (de Bruijn et al. 1989) or estimated by computer modeling using substituent constants (Hansch and Leo 1979; Karickhoff et al. 1991).

The K_{ow} is the dominant physical parameter that explains a substantial amount of partitioning behavior exhibited by PAHs in the environment. A list of K_{ow}s for 24 of the PAHs more commonly measured, illustrating the range of values encountered, is compiled in Table 1. Because K_{ow}s are usually very large numbers, they are more conveniently expressed in logarithmic form; for example, a log K_{ow} of 5.5 is actually ($10^{5.5} = 316,228$). From the least hydrophobic compound, naphthalene, to the most hydrophobic compound on this list, benzo[g,h,i]perylene, the range in K_{ow}s is approximately 3.7 orders of magnitude, or 5000 fold.

Another useful partition coefficient is the ratio between PAH concentrations in sediment organic carbon and water (K_{oc}), which is determined by the concentration of PAH per gram of organic carbon in sediment divided by the concentration of PAH in either overlying or interstitial water (see Appendix A for the formula). Interstitial water, also known as porewater, surrounds sediment particles and is found under the sediment surface. The numerator of this equation is known as Sed_{oc}, the organic carbon-normalized sediment concentration of the PAH, which is used in many calculations. For example, this coefficient is useful for predicting the amount of waterborne PAH for a given sediment concentration under equilibrium conditions. The K_{oc} for a PAH may be predicted with K_{ow}, and the prevailing theory is that K_{oc} is approximately equal to K_{ow} (Di Toro et al. 1991).

The simple characteristic of partitioning between water and sediment by

Table 1. Octanol–water partition coefficients for commonly measured polycyclic aromatic hydrocarbons in environmental samples.

Chemical	MW	Rings	Log K_{ow} 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Mean	sd	sem
Naphthalene	128.2	2			3.30		**3.35**		3.40	3.30		3.35	3.36		3.35	3.45	3.36	3.18	3.36	3.34	0.07	0.02
1-methylnaphthalene	142.2	2					3.87		3.87	3.95		3.87							3.84	3.88	0.04	0.02
2-methylnaphthalene	142.2	2			4.11		3.86		3.86	3.90		3.86							3.86	3.91	0.10	0.04
Biphenyl	153.2	2			3.87	4.01	**3.88**			4.14		3.95		4.06	4.10	4.08	3.63		4.05	3.98	0.15	0.05
Acenaphthylene	152.2	3		4.07									4.08						4.10	4.08	0.02	0.01
Acenaphthene	154.2	3		4.33						4.15		3.92	4.32	3.83					3.95	4.08	0.21	0.10
Fluorene	166.2	2		4.18			4.18			4.27		4.18	4.38		4.18	4.17			4.21	4.22	0.07	0.03
2,6-dimethylnaphthalene	156.2	2					4.31	4.90	4.31	4.32		4.31							4.37	4.42	0.24	0.11
Anthracene	178.2	3		4.45	4.34					4.73		4.54	4.45		4.63	4.59	4.80	4.20	4.53	4.53	0.18	0.06
Phenanthrene	178.2	3		4.46	4.52	4.56	4.46			4.64		4.57	4.46	4.54	4.63	4.42			4.57	4.53	0.07	0.02
2,3,5-trimethylnaphthalene	170.3	2					4.73			4.79		4.90							4.86	4.83	0.09	0.05
1-methylphenanthrene	192.2	3						5.20											5.10	5.15	0.07	0.07
Pyrene	202.3	4		5.32	5.17		4.88			5.12	5.09	5.18	5.30	5.07	5.22	4.93		4.50	5.10	5.07	0.22	0.07
Fluoranthene	202.3	4		5.33		5.16				5.29		5.22	5.53	5.09	5.22				5.10	5.24	0.14	0.05
Chrysene	228.3	4	5.61							6.01		5.79	5.60		5.91				5.71	5.77	0.16	0.07
Benz[a]anthracene	228.3	4	5.61							6.10	6.50	5.90	5.60						5.67	5.90	0.35	0.16
Benzo[e]pyrene	252.3	5																	6.10	6.10	—	—
Benzo[a]pyrene	252.3	5	6.04							6.57		5.98	6.00		6.50				6.30	6.23	0.26	0.12
Perylene	252.3	5								6.53		6.50							6.13	6.39	0.22	0.16
Dibenz[a,h]anthracene	278.4	5		5.97								7.19	6.00						6.71	6.47	0.59	0.34
Benzo[b]fluoranthene	252.3	5		6.57									6.60						6.40	6.52	0.11	0.08
Benzo[k]fluoranthene	252.3	5		6.84									6.85						6.50	6.73	0.20	0.14
Benzo[ghi]perylene	276.3	6		7.23						6.77		7.10	7.00		7.10				7.00	7.03	0.15	0.07
Indeno[1,2,3-cd]pyrene	276.3	6		7.66									7.70						6.92	7.43	0.44	0.31

Log K_{ow} is the $\log_{10}$ octanol–water partition coefficient. Included are the mean, standard deviation (sd), and standard error of the mean (sem) for all data. Data from: 1, Radding et al. (1976); 2, Leo et al. (1971); 3, Karickhoff et al. (1979); 4, de Bruijn et al. (1989); 5, Hansch and Leo (1979); 6, Konasewich et al. (1982); 7, Krishnamurthy and Wasik (1978); 8, Mackay et al. (1980); 9, Means et al. (1980); 10, Miller et al. (1985); 11, Mills et al. (1982); 12, U.S. EPA Athens, slow stir method; 13, Bruggeman et al. (1982); 14, Hammers et al. (1982); 15, De Kock and Lord (1987); 16, D'Amboise and Hanai (1982); 17, early-release prototype version of the SPARC model, Karickhoff et al. (1991). Bold indicates mean of many values from this citation; MW is molecular weight in daltons. No distinction was made between measured and calculated K_{ow}s. Most K_{ow}s for a compound are unique; however, there is the possibility that some entries occur in two or more citations.

neutral organic compounds [such as PAHs and polychlorinated biphenyls (PCBs)] has led to the equilibrium partitioning (EqP) theory, which is used to assess the potential for exposure of animals to neutral organic compounds associated with sediment (Di Toro et al. 1991; Pavlou and Weston 1983). The basis of EqP is that the concentration of a chemical in porewater can be determined if the organic carbon, bulk sediment concentration, and partition coefficient (K_{oc}) are known. Equilibrium between the sediment and water phases is assumed. Recent reports indicate that the model accurately predicts the porewater concentration for most neutral organic compounds (Di Toro et al. 1991). With the ability to predict porewater concentrations, toxicity from sediment can be predicted by employing the large acute-response, water-quality database that has been generated over the years for various species in water-only tests. Of course, this is valid only if sediment–water partitioning behavior is as expected. Because EqP is used for predicting porewater concentrations for sediment-quality criteria, reliable and predictable K_{oc}s are essential. This method of predicting sediment toxicity has been proposed as a management tool for contaminated sediments and is currently being investigated by the U.S. Environmental Protection Agency (USEPA) (Di Toro et al. 1991) and corresponding authorities in other countries (van der Kooij et al. 1991); however, not all researchers agree on its utility.

Many authors have found higher than expected K_{oc}s for PAHs due to partitioning behavior that resulted in lower than expected porewater concentrations. One possible explanation is that PAHs from combustion sources behave differently from other organic contaminants, such as PCBs, in their desorption to water (McGroddy and Farrington 1995; Meador et al. 1995; Prahl and Carpenter 1983; Pruell et al. 1986; Readman et al. 1987; Socha and Carpenter 1987; Varanasi et al. 1985). One study calculated K_{oc}s for several PAHs from naturally contaminated sediments that were 1–2 orders of magnitude higher than predicted (using predicted $K_{oc} = K_{ow}$) (Pruell et al. 1986). Another study by Socha and Carpenter (1987) also found higher than expected K_{oc}s for field-collected sediment in Puget Sound, Washington (U.S.), due to lower than expected interstitial water concentrations. They proposed that these low interstitial water concentrations resulted from combustion-derived PAHs that exhibited strong particle sorption. In the same study, these authors found K_{oc}s as expected for PAHs in nearby creosote-contaminated sediment. Meador et al. (1995) also found K_{oc} partition coefficients for PAHs in laboratory exposures using field-collected sediment to be higher (about 2 orders of magnitude) than predicted, especially when free PAH was considered. Similarly elevated K_{oc}s were found by McGroddy and Farrington (1995) in their study of Boston Harbor (U.S.) sediments and attributed these results to reduced partitioning by combustion PAHs.

These studies finding greater than expected partition coefficients, caused by lower than expected porewater concentrations, examined sediments

from different geographical locations of many square kilometers; hence, these measured K_{oc}s are probably representative of sediments from urbanized coastal areas around the world. One consequence of reduced partitioning would be the erroneous prediction of higher water concentrations, which when used to characterize a test sediment would lead to an underestimation of the true bioconcentration factor and toxicity response (calculated LC_{50}) for organisms exposed to porewater. Comparison of these values with those generated in water-only tests should help uncover these anomalies.

Many studies of PAH partitioning report porewater concentrations as expected (e.g., $K_{oc} = K_{ow}$); however, most of these studies were performed by spiking sediment in the laboratory with individual chemicals and then allowing them to come to equilibrium with porewater, usually within a few weeks. In contrast, field-contaminated sediments appear to behave differently from contaminated sediments prepared in the laboratory. Because of this apparent disparity, it may be necessary to measure neutral organic compounds in porewater directly to estimate sediment toxicity and bioaccumulation for situations in which porewater exposure is important. Thus, many additional studies with field-collected sediments are needed to assess the utility of EqP as a management tool.

Dissolved organic carbon (DOC) in porewater can enhance total dissolved concentrations of hydrophobic compounds (Brownawell and Farrington 1986; Chiou et al. 1986; Di Toro et al. 1991; Socha and Carpenter 1987) and, in general, PAHs sorbed to DOC are not available for uptake by organisms (McCarthy et al. 1985). Because of the strong sorption of PAHs (mainly HPAHs) by DOC, which are composed mainly of organic acids such as humic and fulvic compounds, the concentration of these contaminants in porewater will be higher than predicted in water without DOC. These higher than predicted total porewater concentrations lead to lower than predicted partition coefficients (K_{oc}). If the partition coefficient is expressed in terms of the free chemical, which is unaffected by DOC (Di Toro et al. 1991), the partitioning behavior may be closer to expectation and the variability between sediments may be reduced.

III. Occurrence in Water and Sediment

PAHs are ubiquitous in the marine environment and occur at their highest concentrations near urban centers of human populations (LaFlamme and Hites 1978). They are derived from petroleum sources, combustion products, and natural synthesis by organisms (LaFlamme and Hites 1978). Taken together, PAHs are considered a signature of human activity as a result of industrial processes; however, localized accumulations may result from natural events such as forest fires, volcanism, and petroleum seeps. It is generally believed that if substituted PAHs, including alkyl derivatives, predominate, the source of the PAHs is petroleum based. If, however, the unsubstituted PAHs (especially the HPAHs) predominate, then it is more

likely that the PAHs come from combustion processes. One of the major sources of petroleum hydrocarbons is oil spills, and much research is concerned with the fate and effects of the major aromatic hydrocarbons in oil. Petroleum-based PAHs find their way into the coastal environment through accidental releases of petroleum products and infusion from natural seepage. Combustion-derived PAHs enter the marine environment through aerosol infiltration from combustion of various materials, both natural (such as forest fires) and anthropogenic (e.g., fossil fuel combustion). Naturally derived PAHs can also come from algae and plants (Borneff et al. 1968; Hancock et al. 1970).

It is known that contaminants accumulate in fine-grained sediments (Marcus et al. 1988; Olsen et al. 1993; Stainken et al. 1983) and that PAHs accumulate in sediments because of their hydrophobicity and partitioning to organic carbon-coated particles (Means et al. 1980). Fine-grained or muddy sediments are a characteristic of estuaries, and many species reside in these important habitats. When anthropogenic activities such as sewage disposal, manufacturing, shipping, and recreational boating occur near estuaries, contaminants such as PAHs can accumulate in the sediments to levels much higher than those found in more pristine areas (Farrington et al. 1983; Marcus et al. 1988). The concentrations of PAHs in sediment can range from a few ng/g (ppb) to very many μg/g (ppm) (Eisler 1987; Neff 1979; O'Connor 1991; Wade et al. 1988, 1994). Bjørseth et al. (1979) reported summed PAH concentrations up to 56.65 μg/g (dry weight) near a smelter in Norway, with several individual HPAHs at concentrations greater than 2 μg/g, and Wade et al. (1988) reported PAH concentrations in sediments from the Gulf of Mexico ranging from below detection (5 ng/g) to 36.7 μg/g (dry wt). One study reported that the Elizabeth River system in the Chesapeake Bay area had the highest PAH contamination of any estuary in the world, with total PAHs in sediment up to 170 μg/g (dry wt) (Huggett et al. 1987). Higher concentrations have been reported by Collier et al. (1986) for Eagle Harbor, Washington, where total PAHs in sediment were up to 1.35 mg/g (dry wt), and by Sirota et al. (1983), who reported 2.83 mg/g (dry wt) of total PAHs in sediment at one sample site near a coking plant in Nova Scotia, Canada. Several reviews have presented detailed information regarding the occurrence and sources of PAHs in the environment (Eisler 1987; Mearns et al. 1991; McElroy et al. 1989; Neff 1979, 1985).

The qualitative profile of PAHs in soil and sediments from various locations (United States, Africa, South America) is remarkably consistent between locations, which has been attributed to combustion sources (LaFlamme and Hites 1978). Additional studies of PAHs in sediments have also found that combustion PAHs predominate (Bieri et al. 1986; Hites et al. 1980; Readman et al. 1987). On a finer scale, Barrick and Prahl (1987) found regional sources of combustion-derived PAHs in Puget Sound, Washington, in addition to major inputs from runoff and atmospheric

fallout from urban areas. A predominance of HPAHs was found in tidal sediments around the Washington, D.C. area, indicating probable combustion sources; however, one area contained LPAHs in the range of 60%–90% of total PAHs, indicating a petroleum source (Wade et al. 1994).

Concentrations of PAHs vary on both long and short temporal scales in estuarine areas. On a temporal scale of decades, Hites et al. (1980) found very large increases in total PAHs over time in a river estuary in southern Rhode Island. They noted that total PAH concentrations were relatively low ($\approx$ 120 ng/g) from 1820 to 1900, then increased dramatically, peaking at 14 μg/g in the 1950s (Fig. 1). They attributed the decline after this time to the advent of oil and natural gas as energy sources for home heating, which had been dominated by coal. This temporal trend of PAHs in sediments has been shown in other studies as well (Grimmer and Böhnke 1975; Wenning et al. 1994), with combustion-derived PAHs in sediments from urban areas showing a maximum around the 1950s and pre-1900 concentrations well below 1 μg/g. Temporal differences on a shorter time scale also occur. Stainken et al. (1983) found the highest concentrations of extractable hydrocarbons from sediments in the Raritan Bay, New York area during the spring and summer seasons, possibly due to the spring runoff and thus higher loading to coastal sediments.

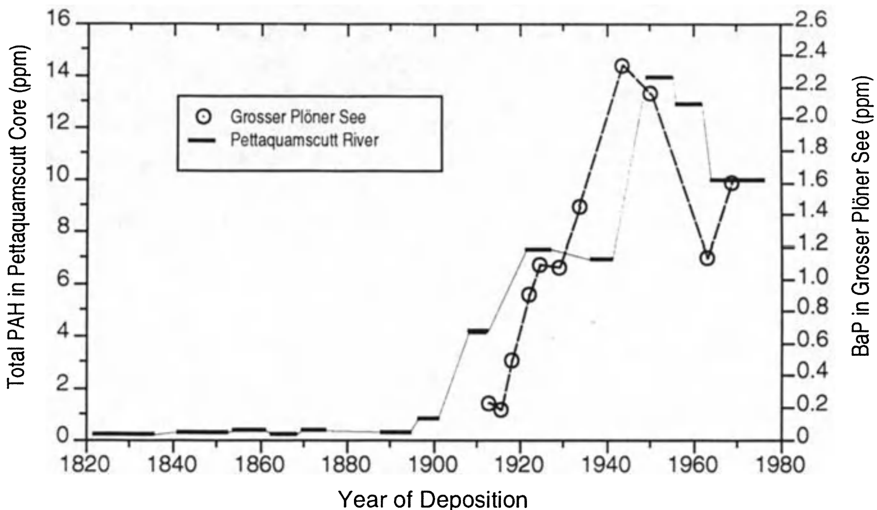

Fig. 1. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in sediment cores over time: total PAH abundance in sediment core sections from Pettaquamscutt River, Rhode Island, U.S.A. and benzo[a]pyrene concentrations in cores from Grosser Plöner See (NW Germany). [Reprinted from Geochimica et Cosmochimica Acta Vol 44, Hites RA, LaFlamme RE, Windsor JG Jr., "Polycyclic aromatic hydrocarbons in an anoxic sediment core from the Pettaquamscutt River (Rhode Island, U.S.A.)," pp 873-878. Copyright (1980), with kind permission from Elsevier Science Ltd., The Boulevard, Langford Lane, Kidlington 0X5 1GB, UK.]

Environmental Fate of Hydrocarbons. A few studies have shed light on the fate of hydrocarbons in the marine environment. In coastal marine sediments, PAHs accumulate from urban sources and are converted to intermediate products and CO_2 by metabolism, photooxidation, and other processes. In their Controlled Ecosystem Pollution Experiment (CEPEX), Lee et al. (1978) concluded that hydrocarbons were deposited in the sediment by falling particles, although they did not specifically study the direct uptake of waterborne PAH by sediment. Their results show that in the first few days of a one-time addition of oil that the sediment concentration of naphthalenes was high and then steadily decreased to below detection by day 17, whereas anthracene, fluoranthene, benz[a]anthracene, and benzo-[a]pyrene concentrations in the sediment steadily increased over the 17 d of the experiment. It was also proposed by Lee et al. (1978) that hydrocarbons from an oil slick that are smaller than 15 carbons volatilize quickly and only to a limited degree for those with 15–25 carbons. They concluded that most LPAHs were removed primarily by microbial degradation and that HPAHs were removed by photooxidation and sedimentation. Another study has shown that when oil is added to the water column, total hydrocarbons rapidly decrease in the water but aromatic hydrocarbons increase in the sediment (Gordon et al. 1976).

The degradation of PAHs can be affected by redox state, temperature, nutrient content, sediment structure, and biological activity in sediment (Gardner et al. 1979). Hinga et al. (1980) examined the fate of benz[a]anthracene in a marine mesocosm system [Marine Ecosystems Research Laboratory (MERL) Kingston, RI, USA], and found that after 216 d, only 21% of the original amount added was parent compound; the rest was CO_2 and intermediate products. Another study found removal rates of 1%–3% wk^{-1} for anthracene, fluoranthene, benz[a]anthracene, and benzo[a]pyrene added to sediments ranging in texture from medium sand to silt-clay sand (Gardner et al. 1979). These and other authors (Gordon et al. 1978; McElroy et al. 1990) found significant enhancement to the rate of degradation of PAHs when deposit-feeding polychaetes were present.

Microbial oxidation of sediment-associated PAHs can be minimal. Herbes and Schwall (1978) found that for PAHs in contaminated freshwater sediments the turnover times (1/rate constant) increased while the rate constants and transformation rates ($\mu g\ g^{-1}\ hr^{-1}$) decreased with increasing number of aromatic rings (increasing hydrophobicity). For example, they determined the turnover times for naphthalene, benz[a]anthracene, and benzo[a]pyrene to be 7 hr, 400 d, and 3.3 yr, respectively. Furthermore, they reported that HPAHs in sediments that had been contaminated for years were resistant to microbial transformation, even with an assumed bacterial population that was capable of transforming LPAH compounds. In their study, benz[a]anthracene and benzo[a]pyrene were almost totally refractory to microbial oxidation. This pattern, in conjunction with higher solubility for LPAHs, may explain why many sediments are enriched with HPAHs.

Additional studies indicate that PAHs may be persistent in sediment. Of several PAHs studied by Lee et al. (1978), only naphthalenes were significantly degraded by microbes (at a rate of up to 5% d^{-1}). In one study of a salt marsh (Buzzards Bay, MA) that was heavily impacted by an oil spill, aromatic hydrocarbons persisted in the sediments for at least 4 yr after the event (Burns and Teal 1979). These results give an indication of the throughput of PAHs in the marine environment, suggesting that the LPAHs are probably not persistent, whereas the results for HPAHs are less clear. Future work determining such persistence of PAHs in sediment under different conditions, especially redox state, will help immensely in understanding and predicting the mass balance and the relative proportions of these compounds in impacted ecosystems.

IV. Factors Controlling Bioavailability to Marine Organisms

As discussed earlier, the proportion of the total contaminant concentration that is available for uptake by organisms defines the bioavailable fraction, and organic carbon is the main variable that controls this fraction for PAHs. For equal sediment concentrations of a hydrophobic series of PAHs in a sediment–water system (ignoring sorption by DOC in the water), as the hydrophobicity of PAHs increases, the ratio of water to sediment concentrations of PAH will decline. One study of PAH uptake found that of 15 HPAHs measured, pyrene dominated (up to 90% of total concentration) in water and algae (*Fucus vesiculosus* and *Enteromorpha intestinalis*); however, in surficial sediments, pyrene accounted for only 9% of the total HPAHs (Kirso et al. 1990). These results suggest that the greater water solubility of pyrene and relatively low sorption to DOC resulted in higher bioavailability of pyrene compared to all other HPAHs measured in this study.

Many authors have shown reduced bioavailability of PAHs to aquatic organisms because of sorption to DOC, which is probably important only for very hydrophobic compounds (i.e., HPAHs) at natural DOC levels (Landrum et al. 1985, 1987; Leversee et al. 1983; McCarthy 1989). Partitioning between DOC and porewater (K_{doc}) is expected to be similar to that observed between octanol and water (K_{ow}) and is reported to vary between 1.0 K_{ow} and 0.1 K_{ow} for PAHs (Caron and Suffet 1989; Gauthier et al. 1987; Landrum et al. 1985). Several studies have shown variable K_{doc}s, which could be related to DOC composition (Caron and Suffet 1989; Gauthier et al. 1987; Landrum et al. 1984). For example, Landrum et al. (1985) reported that a natural porewater with low DOC had a higher K_{doc} for benzo[a]pyrene than porewater with high DOC. Meador et al. (1995) found that the variability in HPAH bioconcentration factors (BCFs) between sites was reduced when two K_{doc}s were used (0.1 K_{ow} for high DOC sites and a stronger K_{doc} (= 1.0 K_{ow}) for low DOC sites) to calculate free PAH concentrations in porewater. Because of this reduction in variability, they concluded that DOC may have been qualitatively different (such as having

variable humic acid content) between high and low DOC sites or that HPAHs exhibited stronger sorption when DOC concentrations were low. Support for the latter hypothesis of stronger affinity at low DOC levels comes indirectly from Hunchak-Kariouk (1992), who found higher K_{doc}s for tetrachlorobiphenyl in oxic porewater compared to that observed in anoxic porewater. In the study by Meador et al. (1995), the low-DOC porewaters producing the higher K_{doc}s were from sites with coarse sediment, which generally tend to have porewater that is more oxic than that found in sediments with a higher percentage of fine particles.

Few studies have examined the effects of DOC sorption on the uptake of PAHs in marine organisms. When the concentrations of PAHs in porewater were expressed in terms of the free fraction, the BCFs for the amphipod *Rhepoxynius abronius* (a nondeposit feeder) were closer to expected values (Figure 2), indicating that the free PAH in porewater was the bioavailable fraction (Meador et al. 1995). Another study found that Atlantic salmon exposed to benzo[a]pyrene in water with a total organic carbon

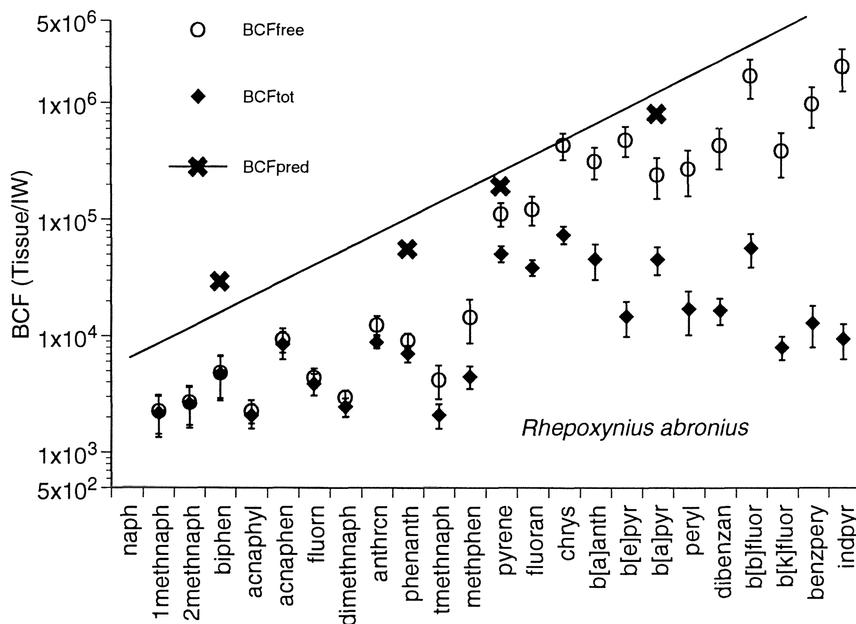

Fig. 2. Bioconcentration factors (BCF) for *Rhepoxynius abronius* determined with dry weight divided by interstitial water concentration. *Closed symbols*, calculated with total PAH in interstitial water; *open symbols*, BCF calculated with free PAH in interstitial water (where $K_{doc} = K_{ow}$); large X is the predicted BCF (data from Landrum 1988; Landrum et al. 1994). Chemicals on abscissa increase with K_{ow}. Bars are mean and standard error of the mean at d 10 of exposure. See Appendix for chemical abbreviations. (Redrawn from Meador et al. 1995.)

(TOC) of 22 mg C/L exhibited a reduced BCF for this compound that was about 50% of that in fish exposed to control water with 3.6 mg C/L (Figure 3) (Johnsen et al. 1989). Conversely, a study of uptake by the clam *Mercenaria mercenaria* determined that the presence of DOC (3.1 vs 0.7 mg C/L) had little effect on phenanthrene accumulation in tissue (Boehm and Quinn 1976). Obvious differences between these two studies suggest that the quality and quantity of DOC and PAHs used will have an influence on the uptake of PAHs by marine organisms. Both studies are environmentally relevant because the low-DOC treatment reflects concentrations found in the water column of coastal waters and the high-DOC treatment more closely approximates those values found in porewater (Brownawell and Farrington 1986; Landrum et al. 1987; Lyons et al. 1979; Meador et al. 1995). Unlike many freshwater systems, most coastal marine waters do not have elevated or variable DOC concentrations, unless the interstitial water is examined. Consequently, sorption of PAHs by organic carbon in open water is not a major concern; however, in some estuarine areas, DOCs may be elevated and consequently alter PAH bioavailability. Some sediment porewaters can have DOC in the 100 mg C/L range and consequently HPAHs may show reduced availability to organisms. Recent research indicates that the bioavailability of LPAHs may not be greatly affected by environmental concentrations of DOC because only a small fraction will be sorbed (Landrum et al. 1987; McCarthy et al. 1985; Meador et al. 1995).

The bioavailability of PAHs to sediment ingesters is an area of research that has received little attention. A study of the bioavailability of ^{3}H-benzo[a]pyrene in sediment to a deposit-feeding polychaete (*Abarenicola*

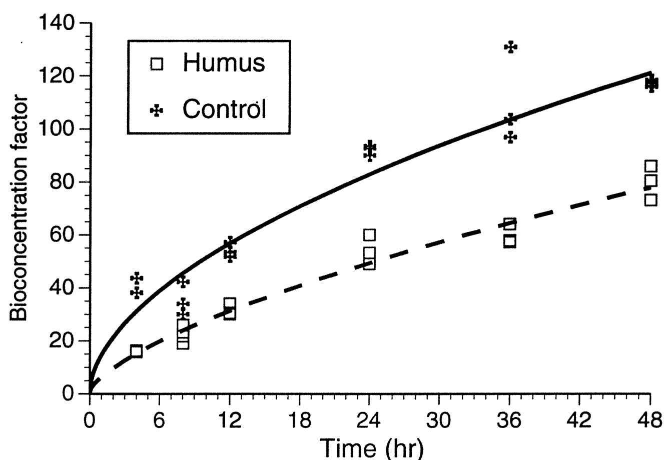

Fig. 3. Bioconcentration factors of benzo[a]pyrene in Atlantic salmon from control water and treatment water with 21.7 mg C/L organic carbon during uptake period. (Redrawn from Johnsen et al. 1989 with permission from Elsevier Science B.V.)

pacifica) found that bioaccumulation of this compound was higher in sediment containing low TOC (0.3%) compared to sediment with high TOC (1% and 2%) even though organic carbon-normalized sediment concentrations and interstitial water concentrations of benzo[a]pyrene were essentially the same in all three test sediments (Weston 1990). This was explained by the polychaete's ability to select fine particles, which were enriched with organic carbon and benzo[a]pyrene. Another factor that may have contributed to the higher tissue concentrations is the amount of free benzo[a]pyrene (nonsorbed) in interstitial water. In the test sediment with low TOC, the porewater DOC levels may also have been low, and because DOC has been shown to sorb and substantially reduce the bioavailability of HPAHs, the porewater with the lowest DOC would have the highest amount of bioavailable (free) benzo[a]pyrene. Another study (Meador et al. 1995) utilizing a sediment-ingesting polychaete (*Armandia brevis*) concluded that while the partitioning of PAHs into interstitial water was depressed by approximately 1–2 orders of magnitude, PAH accumulation (BAF_{loc} adjusted for metabolism) by the polychaete was closer to the expected value of 1.7 (see Lee 1992). Hence, even though hydrophobic PAHs were more tightly bound to the sediment particles in an aqueous environment, when these same sediment particles were ingested by the polychaete the PAHs were more labile and readily desorbed into the gut mucosa.

One study has examined the influence of different organic materials on the partitioning and toxicity of sediment-associated fluoranthene (DeWitt et al. 1992). This study concluded that the toxicity response of an infaunal amphipod (*Rhepoxynius abronius*) to fluoranthene-spiked sediments ranged only twofold for such diverse organic amendments as invertebrate feces to fresh eelgrass (*Zostera marina*). Additional research that examines other PAHs (especially the very hydrophobic) and species with different routes of uptake (e.g., deposit feeding) will be useful to those attempting to predict effects based on organic carbon-normalized sediment concentrations.

A few studies have found differential partitioning and bioavailability, depending on the type of PAH and their history of association with sediment. For example, Varanasi et al. (1985) demonstrated that recently added (spiked) PAH in sediment was more bioavailable to organisms than the PAH in field-contaminated sediment. Farrington et al. (1983) pointed out that PAHs from oil spills may be less strongly sorbed by sediment than pyrogenic PAHs and hence more available to organisms. McGroddy and Farrington (1995) also reported that pyrogenic PAHs such as phenanthrene are tightly bound to sediment and only 0.2%–5% is available for equilibrium partitioning. These lower water concentrations can produce an accumulation in organisms that is much lower than predicted.

The reader is referred to numerous review articles on the bioavailability and bioaccumulation of chemical contaminants in aquatic organisms including, but not limited to, those by Staples et al. (1985), Knezovich et al.

(1987), Karickhoff and Morris (1987), Rodgers et al. (1987), Adams (1987), McCarthy (1989), McElroy et al. (1989), Landrum and Robbins (1990), Di Toro et al. (1991), Schrap (1991), Lee (1991), Opperhuizen (1991), Lee (1992), and Hamelink et al. (1994). These reviews provide additional information on the factors that affect bioavailability of PAHs and other xenobiotics by aquatic organisms.

V. Uptake and Elimination by Marine Organisms

The body burden of a PAH is determined by the balance between uptake and elimination, each of which can be influenced by many factors. The determination of uptake and elimination can be as simple as measuring tissue concentrations at two different time points; however, the rate at which these processes occur is much more informative and can be used to compare species differences as well as predict steady-state accumulation. Uptake is controlled externally by the partitioning behavior of the contaminant (between sediment, water, and food) and internally by the organism's behavior and physiology. The biological processes that can influence uptake include organism size, growth rate, membrane permeability, ventilatory rate, extraction efficiency, ingestion rate, gut residence time, and osmoregulation. Some of these processes are intrinsic to a species, others are interdependent (e.g., ingestion rate and growth rate), and many may be impacted by environmental factors such as temperature, oxygen content, pH, and salinity (Landrum 1988; McKim 1994; Thomas and Rice 1982). Some of these factors may also be affected by changes in organismal behavior, endogenous and seasonal rhythms, nutritional quality, and stress.

Elimination can be accomplished by passive diffusion when external concentrations are lower than internal concentrations favoring outward flux and by enzymatic pathways that convert hydrophobic parent compounds to more polar metabolites that can be more readily excreted by those taxa that possess a kidney or kidney-like organ (vertebrates and invertebrates such as annelids, molluscs, and arthropods). Conversion of the hydrophobic PAH to a more polar metabolite will decrease its ability to diffuse through the gill membrane, thus favoring the excretory route. The rate of elimination may be affected by environmental factors such as temperature and salinity, and by physiological factors, including reproductive state, age, sex, stress, and enzyme induction, in addition to such factors as route of uptake, chemical hydrophobicity, and exposure history.

The uptake clearance constant (k_1), under conditions of constant environmental exposure, in combination with the elimination rate constant (k_2) is useful in determining the steady-state bioconcentration factor and hence the resulting toxic response (see Appendix for formulae). The uptake clearance constant has units of mL g^{-1} hr^{-1} and describes the volume of source compartment scavenged of a contaminant per gram of animal per unit time. A true uptake rate constant describes the fractional change in the water

concentration per unit time (e.g., hr^{-1}) and depends on the relative sizes of the animal and water compartments (Barron et al. 1990; Landrum et al. 1992).

Much confusion exists about k_1 because of the terminology. Many researchers that report k_1 call it an uptake rate constant; however, they are actually describing the uptake clearance constant of mL g^{-1} hr^{-1}, which is a rate function because it does include a normalization for time. The elimination rate constant describes the fractional elimination from an organism and is expressed in terms of reciprocal time (e.g., hr^{-1}). Both uptake and elimination are first order because the flux into and out of the organism depends on the chemical activity (concentration) (Landrum et al. 1992). The present review uses the uptake clearance constant (k_1) that is determined under conditions of constant exposure and the elimination rate constant (k_2) that is determined without the contaminant present. These toxicokinetic values are conditional in that they are defined for environmental (e.g., temperature, salinity, pH, oxygen concentration) and physiological (e.g., organism size, dietary input, stress) variables. Some of the factors controlling uptake and elimination may be species specific; however, most other factors can cause differences in uptake between individuals within a species. The uptake clearance constant will differ depending on whether the tissue concentration is expressed in terms of wet or dry weight, whereas for determination of k_2 the same value will be obtained whether the tissue concentration is expressed on a wet or dry weight basis.

The conceptual framework for toxicokinetic models (vis-á-vis equilibrium models) used in aquatic toxicology has been reviewed by Barron et al. (1990) and Landrum et al. (1992). Landrum et al. (1992) provides mathematical formalisms and explains how compartment-based models (rate constant, clearance volume, and fugacity models) and physiologically based models (physiological-based pharmacokinetic and bioenergetics-based models) are used. The biggest advantage of the kinetic models is that they are not constrained by the assumption of equilibrium conditions. Landrum and colleagues have also addressed the toxicokinetics of contaminants associated with sediment (Landrum 1989; Landrum et al. 1992). They define uptake clearance (k_s) of sediment-associated contaminants as the amount of sediment (g dry wt) cleared per gram of organism per hour, which includes contributions from interstitial water and sediment ingestion or contact. Uptake clearance also incorporates information about the bioavailable fraction of a contaminant, and a ratio of this parameter to the elimination rate constant (determined with contaminant exposure present) can be used to estimate the bioaccumulation factor (BAF) ($= k_s/k_e$) for sediment-dwelling organisms. The reader is referred to the many papers of Landrum and colleagues (see citations), who have made important contributions to the understanding of PAH toxicokinetics and bioaccumulation in freshwater invertebrates. Many of the principles described in their papers also apply to marine organisms.

A. Uptake

Uptake of some contaminants (e.g., metals), in the presence of seemingly high environmental concentrations, is not easily determined; however for many organic contaminants, evidence of uptake in contaminated areas is usually observed. For those organic contaminants that are metabolized, uptake and the potential for accumulation are more difficult to determine, although the quantification of metabolites will greatly improve assessment. Even at extremely low environmental concentrations of PAHs (i.e., certainly below detection by modern analytical instruments), aquatic organisms will accumulate PAHs because partitioning to the organism is thermodynamically favored and the bioconcentration factors are quite high (generally 10^2–10^6). For example, when water concentrations of PAHs were in the low ng/mL range, zooplankton (primarily *Pseudocalanus minutus*) accumulated body burdens of anthracene, fluoranthene, benzo[a]pyrene, and benz[a]anthracene in the low μg/g range after 4 d of exposure (Lee et al. 1978).

The pattern of synergism and antagonism in PAH uptake, in the presence of other contaminants, has been rarely studied. One study found inhibition in uptake of naphthalene by oysters (*Crassostrea virginica*) when present with PCBs and benzo[a]pyrene (Fortner and Sick 1985). When oysters were exposed to seawater with only naphthalene, uptake of both dissolved and particulate naphthalene produced tissue burdens of this compound that were always slightly higher (except for labile palps and mantle, which were both substantially higher). Conversely, these authors observed that naphthalene and PCBs had no effect on benzo[a]pyrene uptake. In contrast, Stein et al. (1984) observed higher body burdens of benzo[a]pyrene metabolites in English sole (*Parophrys vetulus*) exposed to sediment containing benzo[a]pyrene and PCBs than in fish exposed to sediment containing only benzo[a]pyrene. This may have occurred because enzyme induction led to greater metabolism and thus to higher amounts of metabolites that were not immediately excreted.

1. Routes of Uptake. A number of studies have addressed the routes by which contaminants enter the organism. The route of uptake may not be important under equilibrium conditions, as long as all compartments (e.g., tissue, sediment, water) are in equilibrium. Theoretically, if an aquatic organism is placed in an environment in which sediment, water, and its prey are all at equilibrium, then that organism will come into equilibrium with all of those components (Bierman 1990). Thus, from a thermodynamic perspective, it can be argued that the route of uptake is unimportant in the determination of body burden under equilibrium conditions. Caution is warranted because many factors are important here. When sediment–water partitioning is not as expected or prey body burdens are far below predicted equilibrium concentrations, the route may be very important in determining the steady-state body burden of the species under consideration.

Furthermore, when experiments are conducted under short-term, nonequilibrium conditions, the route of contaminant uptake for the organism can have a major influence on tissue concentration. One should be careful of extrapolations in these short-term tests because the organism may not have sufficient dietary input (is not given food or exhibits abnormal feeding behavior); hence, the dietary route of uptake for a species may not be adequately assessed.

In the pelagic environment, fish and invertebrates may take up PAH contaminants from water through diffusion across their gills and integument and via their diet. Fish and invertebrates associated with sediment, either living underneath (infaunal) or on top (epibenthic) of it may also accumulate PAHs via diffusion of waterborne PAHs across their gills and integument, by dietary uptake (including ingestion of plants, animals, or sediment), or by contact with the sediment allowing diffusion through the integument. Because animals engage in many activities (e.g., ventilate their gills, eat other animals, and burrow through sediment), multiple routes of uptake are expected. Some recent studies indicate that the route of uptake may be a result of how the contaminant partitions rather than a consequence of the organism's habits.

When considering the route of uptake, there are two important factors to consider: the percentage acquired from each route of uptake and the partitioning behavior of the compound. Even though a very small percentage of a hydrophobic PAH will be dissolved in solution, a species that filters large quantities of water will receive a large proportion of its body burden from this route of uptake. For example, Pruell et al. (1986) concluded that water was the main source of HPAHs to mussels (*Mytilus edulis*), probably because it is a suspension feeder. These types of studies are complex, with many variables (such as mode of feeding and environmental conditions) that will have a significant effect on the outcome. As aptly noted by Pruell et al. (1986), when making conclusions concerning the route of uptake it is important to limit the extrapolations to the observation at hand.

One theory on bioaccumulation from sediment states that porewater exposure is the main route of uptake for animals that live under the sediment's surface or closely associated with it (Adams 1987; Adams et al. 1985; Knezovich and Harrison 1988; Oliver 1987; Roesijadi et al. 1978a,b). Other studies, however, conclude that ingestion of contaminated sediment can be a major contributor to body burden of hydrophobic compounds (Boese et al. 1990; Lynch and Johnson 1982; McLeese and Burridge 1987; Meador et al. 1995; Weston 1990). In those cases where uptake via water is important, the presence of sediment can markedly reduce accumulation and toxicity of a chemical (Knezovich and Harrison 1988; Knezovich and Inouye 1993; Schrap and Opperhuizen 1990) because of the ability of the sediment to sorb the chemical and reduce its dissolved concentration.

The mechanism by which an organism acquires food will also have a

major impact on the route of uptake of contaminants. In 4-d water-only exposures, the mean tissue concentrations correlated with the volume of water processed by the four species studied (McLeese and Burridge 1987). Tissue concentrations were higher in the mussel (*Mytilus edulis)* and clam (*Mya arenaria)*, which can filter large volumes of water, whereas the shrimp (*Crangon septemspinosa*) and polychaete (*Nereis virens*) were exposed to much smaller volumes of water passing over their surfaces. When these same species were exposed to sediment, the polychaete appeared to have the highest tissue burdens, indicating that sediment ingestion was a more important source; however, this species was analyzed without purging the gut. Without further study of this species, it is debatable whether sediment retained in individuals will lead to a significant overestimation of bioaccumulated compounds. Another study that exposed a nondeposit-feeding clam (*Tapes japonica*) to sediment found accumulation of several (but not all) PAHs in tissues (Obana et al. 1983). They found similar accumulations in clams placed on the sediment surface as well as those placed 10 cm above it, thus indicating uptake via water exposure or ingested suspended sediment.

Other authors have invoked feeding strategy as a possible mechanism to explain the observed patterns of accumulation of PAHs. When exposed to PAH-contaminated sediments in a flow-through bioassay system, a deposit-feeding clam (*Macoma inquinata*) and a sipunculid (*Phascolosoma agassizii*) accumulated more naphthalenes (including methyl- and dimethylnaphthalene) than a suspension-feeding clam (*Protothaca staminea*) (Roesijadi et al. 1978a). These authors concluded that interstitial water was the primary route of uptake for these infaunal species because the deposit feeder would be exposed to contaminants in porewater whereas the suspension feeder would not. In another study, Roesijadi et al. (1978b) found that a deposit-feeding clam (*Macoma inquinata*) took up very little of the LPAH methylnaphthalene from sediment ingestion and that all of its tissue burden could be accounted for by uptake from water. Another study examined routes of uptake by comparing an infaunal nondeposit-feeding amphipod (*Rhepoxynius abronius*) (which was suspected to have inadequate dietary input during laboratory exposure) and a deposit-feeding polychaete (*Armandia brevis*) and found similar accumulations of LPAHs but large differences in HPAH accumulation (Meador et al. 1995). Even though these species had different feeding modes, they exhibited similar BCFs for the LPAHs but a large difference for the HPAHs. This study suggests that deposit-feeding and nondeposit-feeding infaunal invertebrates will acquire most of their body burden of LPAHs through porewater regardless of feeding strategy; however, ingestion of sediment or food may be the dominant route of uptake when hydrophobic compounds exceed a log K_{ow} of approximately 5.5.

One study indicated that uptake of LPAHs occurred mainly through the dietary route. Corner et al. (1976) concluded that adult female copepods

(*Calanus helgolandicus*) accumulated more naphthalene after 24 hr when uptake was by diet as opposed to exposure in water without prey. Assessment of the importance of dietary accumulation is difficult in aquatic systems because prey can release compounds to the water, thus confounding the experimental design of assessing only dietary input. For most aquatic organisms, we would expect dietary uptake of LPAHs, such as via sediment ingestion, to have a minor impact on tissue concentrations for these compounds when prey and water are at steady state with each other.

When an organism is in contact with sediment but does not ingest it, equilibrium or steady-state tissue concentration may be very different, reflecting only the organism's interaction with overlying water. It may be possible that a clam living in the sediment experiences little exposure because it filters overlying water that may never be in equilibrium with the sediment. Support for this comes from Foster et al. (1987), who found that a deposit-feeding clam (*Macoma balthica*) accumulated PAH, whereas a filter-feeding clam (*Mya arenaria*) did not; however, the metabolism of these compounds also needs to be considered.

The presence of organisms in the sediment has been shown to increase PAH concentrations in overlying water, which may be an important factor in assessing bioaccumulation for some species. For example, it has been shown that the presence of a tubiculous polychaete (*Nereis virens*) can enhance the flux of sediment-sorbed benz[a]anthracene to the water column (McElroy et al. 1990). This increased flux to the water column could elevate tissue concentrations in those animals that take in PAHs through gill membranes by ventilating overlying water.

The importance of ventilation in uptake has been examined by Landrum (1988), who determined that the uptake clearance for heat-killed animals was only about 15% of that observed for live animals exposed to PAHs (biphenyl, anthracene, and benzo[a]pyrene) in water. This work indicates that uptake from active ventilatory movement, as compared to that from simple static diffusion, is necessary to achieve high tissue concentrations in this species and probably others that move water over gill structures for respiration.

Xenobiotic metabolism often makes PAH exposure difficult to determine; however, gut contents may indicate that an animal has at least ingested these compounds and is probably accumulating some portion. Although Sammut and Nickless (1978) did not find PAHs in gray mullet (*Mugil cephalus*) flesh, they did find phenanthrene, anthracene, pyrene, fluoranthene, chrysene, and benzopyrenes in the gut contents. They determined that these fish were ingesting sediment, which they found to contain ppm levels of aromatic hydrocarbons. Other field studies have also found evidence of PAH exposure in stomach contents of marine organisms (Stein et al. 1995b; Varanasi et al. 1989b).

Because hydrophobic PAHs partition between the different environmental compartments of sediment, water, and tissue to varying degrees, de-

pending on their fugacity, and because organisms have multiple routes of uptake, one may expect differential accumulation from each source depending on the PAH. Such calculations have been made for a freshwater amphipod [*Diporeia* spp. (*Pontoporeia hoyi*)] showing a decreasing contribution to body burden from porewater as hydrophobicity increased (Landrum 1989; Landrum and Robbins 1990). Similar values of percentage contribution from water and diet for these PAHs were hypothesized in a model for contaminant uptake in fish based on respiratory and feeding rates (Chapman 1987). Because it was concluded that the amphipods in the report by Meador et al. (1995) received most of their PAH body burden through porewater, the percentage tissue burden for each PAH in the deposit-feeding polychaete that came from porewater can be determined from the ratio between the two species studied. All three studies agree on the basic pattern of decreasing uptake through water and increasing uptake via the diet as PAH hydrophobicity increases.

The contribution of PAHs from water and dietary sources to tissue burden was examined by modeling. A series of curves showing the percentage uptake from water as a function of hydrophobicity was developed for two different modes of feeding. For this exercise the sediment PAH concentration was set to 1000 ng/g with a TOC of 1%. Further, the K_{oc} was assumed equal to K_{ow} to calculate the equilibrium water concentrations (see Appendix for formulas), and a constant exposure concentration was assumed. Because there is little information regarding the assimilation of PAHs from ventilated water or ingested sediment, a family of curves with a 100-fold range in uptake efficiency was generated. In Figs. 4 and 5, the first value is for ventilatory uptake efficiency and the second value is for sediment uptake efficiency. For example, the first curve, 1–100, is for 1% ventilatory and 100% sediment uptake efficiency. We plotted nine curves to give a range in values, which can also be considered as ratios (i.e., 100–1 will cover all values with a ratio of 100 : 1).

One case considers a suspension-feeding mussel (Fig. 4). Although a large range of values was found for the rate of filtration in the literature, we chose 50 mL min^{-1} or 72 L d^{-1} (Bayne 1976). To model the route of uptake through ingestion, suspended sediment was assumed to be the source of PAHs to mussels. For the amount of suspended sediment that may be found in the water column, 8 mg/L was chosen as corresponding to values measured in Puget Sound, Washington. Hence, because our model mussel filters 72 L d^{-1}, this equals 576 mg of suspended sediment ingested per day. The potential contribution of PAHs from ingested algal cells was ignored to simplify the model. Because algae can metabolize PAHs (Cerniglia and Heitkamp 1989), the amount of PAH that is taken up through ingestion of algal cells may be small. Assumptions aside, the amount of PAH ingested can be adjusted to give another dimension to the model. It should also be kept in mind that the mussel can reject inorganic material and that the rate of filtration and ingestion will vary with organism size

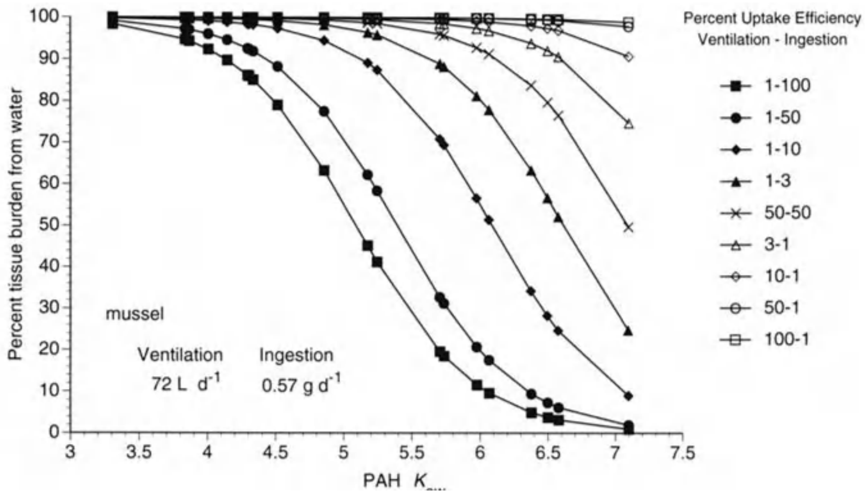

Fig. 4. Model of the relative contribution of sediment ingestion and ventilation routes of uptake to tissue burden for a filter-feeding bivalve. Response variable is the percentage of tissue concentration that would result from water exposure based on a filtration rate of 72 L d^{-1} and an ingestion rate of 0.57 g suspended sediment d^{-1}. Each curve is a combination of percentage uptake efficiency from ventilation (first legend value) and sediment ingestion (second value). For example, 100-1 means that animals assimilated 100% of chemical from ventilatory activity and 1% of ingested chemical.

(Bayne 1976). DOC was ignored for the mussel because levels in coastal areas are expected to be low (2–4 mg C/L). As one can see from the figure, there is a very large range in the percentage of tissue burden from water uptake, which is dependent on the uptake efficiency.

The other case considers an infaunal amphipod that ventilates interstitial water and ingests sediment (Fig. 5a,b). For these curves, we included PAH sorption (K_{doc} = 0.1 K_{ow}) and assumed that the amphipod took up only nonsorbed PAH ($[Water]_{free}$), which was determined with the formula found in the Appendix. Because ventilation data are uncommon, we used values for copepods compiled in Grahame (1983) to estimate this parameter and present two families of curves with two different ventilatory volumes. We used a sediment ingestion rate of 0.06 mg mg^{-1} d^{-1} (wet wt) calculated by Harkey et al. (1994) for a deposit-feeding amphipod. Our model amphipod was 10 mg wet weight, and we set porewater DOC at 100 mg C/L. As seen in the two sets of curves, both uptake efficiency and ventilatory volume have a large impact on the percentage uptake that occurs from water exposure. The result of such an exercise suggests that with multiple uptake routes, variable ventilatory rates, and variable uptake efficiencies, the major route of uptake can change; hence, determining the predominant route may be very difficult. One can imagine that over several days an individual

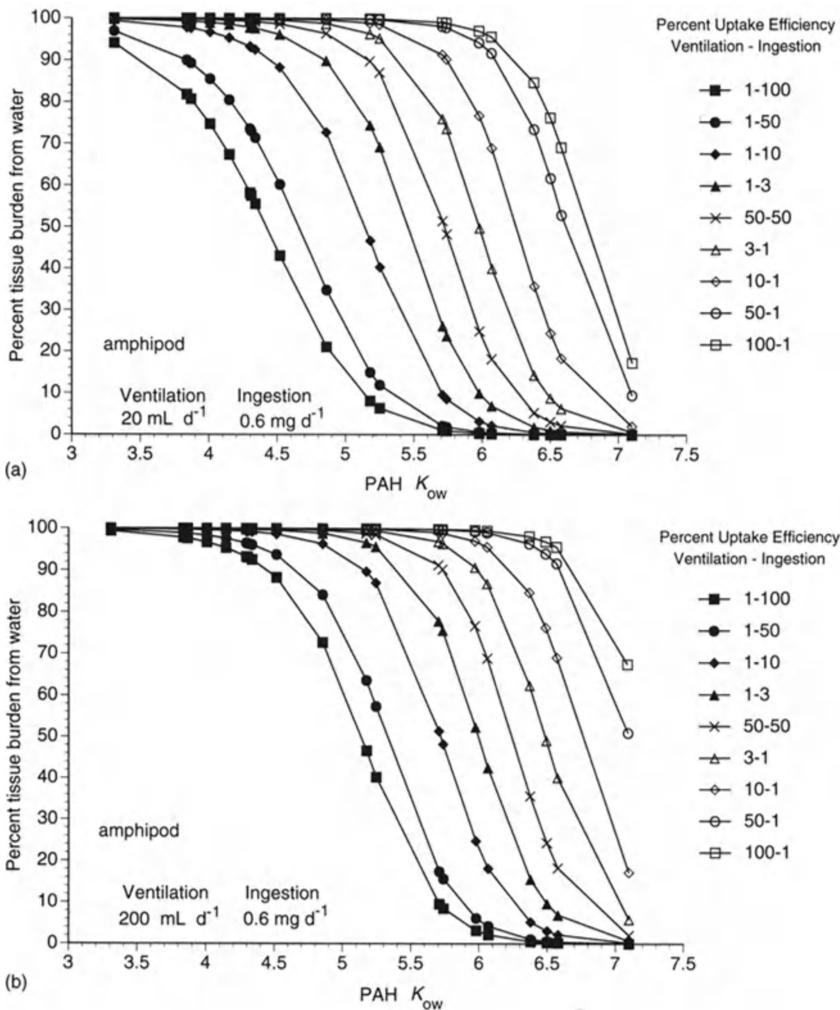

Fig. 5. Model of the relative contribution of sediment ingestion and ventilation routes of uptake to tissue burden for sediment-ingesting amphipod. Response variable is the percentage of tissue concentration that would result from water exposure. (a) Ventilation rate, 20 mL/d; ingestion rate, 0.6 mg sediment/d. (b) Ventilation rate, 200 mL/d; ingestion rate, 0.6 mg sediment/d. Each curve is a combination of percentage uptake efficiency from ventilation (first legend value) and sediment ingestion (second value). For example, 100-1 means that animals assimilated 100% of chemical from ventilatory activity and 1% of ingested chemical.

organism may have variable ventilatory and ingestion activity. Additionally, it should be remembered that equilibrium conditions may not occur; hence, water concentrations will be lower than expected, enhancing the percentage contribution of ingestion to total body burden.

Another route of PAH uptake by organisms to consider is through contact or absorption through the integument; however, very few studies have examined this possible route of uptake. Meador et al. (1995) compared the bioaccumulation of PAHs by two infaunal invertebrates, both of which were in contact with the sediment, and concluded that sediment contact probably contributed little to overall body burden. They observed similar concentrations in tissues for LPAHs but a large difference between the HPAHs, which indicates that any contribution by sediment to tissue burden was probably small. If sediment contact was an important contributor to total body burden, it is unlikely that a mechanism would exist that would allow LPAH uptake by sediment contact in both species to be similar but allow reduced uptake of HPAHs by only one of the species. The exception to this would be if sediment contact was an important source for only LPAHs.

2. Uptake Efficiency. An important area of research concerns uptake efficiency of PAHs from water and dietary sources. By knowing the efficiency of extraction of PAHs from an environmental matrix and the amount of water passing the gills or food ingested, one could attempt to model uptake in a predictive fashion using a bioenergetics-based model (see Landrum et al. 1992). A few studies that have looked at this question show that, in general, assimilation of PAHs from food and water can range from relatively low to almost total extraction. As shown in Figs. 4 and 5, the efficiency of uptake may be very important in determination of the route of uptake, which may influence steady-state body burdens.

Because the ratio of free PAH in water to sediment will decline by orders of magnitude as hydrophobicity increases (Karickhoff et al. 1979; Meador et al. 1995) and the rate of uptake from water for individuals within a species does not appear to vary substantially over a range of PAHs (Bender et al. 1988; Landrum 1988), this may imply, all else being equal, that the uptake efficiency from water does not change significantly over a hydrophobic series of PAHs (except for the very hydrophobic compounds; log K_{ow} > 6.5–7.0) (Konemann and van Leeuwen 1980; McKim et al. 1985; Oliver 1984; Opperhuizen 1991). Support for this comes from McKim et al. (1985), who found consistent gill [trout, *Oncorhynchus mykiss* (*Salmo gairdneri*)] extraction efficiencies (60%) for organic compounds over log K_{ow}s ranging between 3 and 6, indicating that uptake was controlled by aqueous diffusion rates rather than gill membrane permeability. Consistent uptake rates and efficiencies for small fish exposed to chlorinated aromatic hydrocarbons over a wide range of K_{ow}s was also reported by Opperhuizen (1991).

PAHs are assimilated to differing degrees by marine organisms. Diatoms (*Thalassiosira pseudonana*) were found to have more efficient uptake of benzo[a]pyrene (19.1%) from water than clams (*Mercenaria mercenaria*) that ingested the diatoms (5.4%) (Dobroski and Epifanio 1980). Another

study found that crabs (*Callinectes sapidus*) assimilated 2%–10% of the hydrocarbons present in ingested shrimp (Lee et al. 1976). An inverse relationship between the amount of benzo[a]pyrene-spiked sediment ingested and uptake efficiency was found by Weston (1990) in the lugworm (*Abarenicola pacifica*) (Fig. 6). As an individual approached steady state, the accumulation efficiency declined, indicating equal movement into and out of the animal.

Data on uptake efficiencies in freshwater organisms may indicate the types of values expected for similar marine species. Uptake efficiencies of several organic contaminants by zebra mussels (*Dreissena polymorpha*) from the Great Lakes have been found to be high (Bruner et al. 1994), indicating that bivalves are capable of efficiently removing these compounds from algae and sediment. Another study computed the uptake efficiency of ingested radiolabeled benzo[a]pyrene in sediment for a deposit-feeding amphipod (*Diporeia* spp.) to fall between 6% and 33% (Harkey et al. 1994).

Studies of dietary uptake of PAHs in fish generally indicate low uptake efficiency. In their study of dietary absorption in rainbow trout [*Oncorhynchus mykiss* (*Salmo gairdneri*)] fed trout diet, Niimi and Dookhran (1989) found dietary absorption of PAHs ranging from 2% to 32% for compounds such as phenylnaphthalene, acenaphthylene, methylanthracene, triphenylene, and perylene. The lower absorption efficiencies occurred when

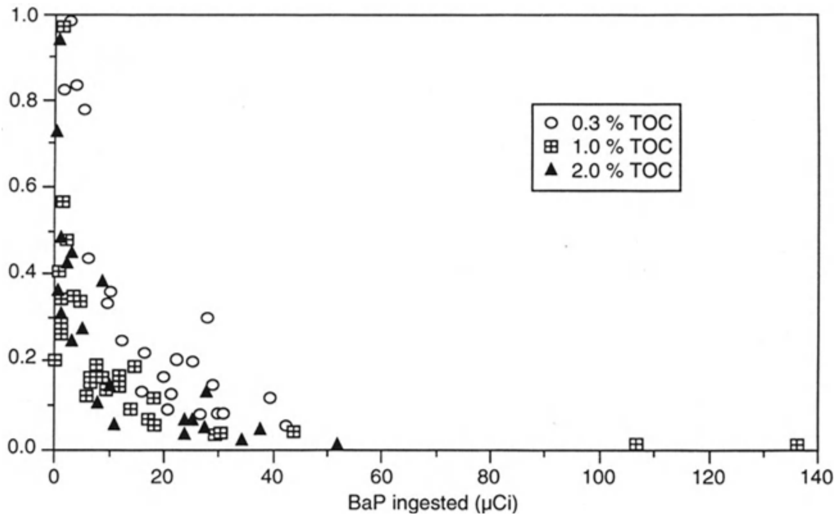

Fig. 6. Accumulation of benzo[a]pyrene (BaP) in the deposit-feeding polychaete *Abarenicola pacifica*. Accumulation efficiency is determined as the proportion of ingested BaP that was retained in the tissues of worms held in low, middle, and high total organic carbon (TOC) treatments. Individuals were sampled after 24 or 96 hr of exposure. (Redrawn from Weston 1990.)

the PAHs exceeded a molar volume of 0.3 nm^3. Additionally, even though similar amounts of benzo[b]fluorene, dibenzo[a,h]anthracene, benzo[ghi]-perylene, and coronene were added to the diet, the tissue burdens were so low after 3 d of feeding that no estimate of dietary absorption could be obtained. Niimi and Palazzo (1986) also reported uptake efficiencies of 14% for fluorene, 4% for phenanthrene, and less than 1% for anthracene, fluoranthene, benzo[a]pyrene, and benz[a]anthracene in rainbow trout [*Oncorhynchus mykiss* (*Salmo gairdneri*)] fed herring oil spiked with PAHs.

These studies indicate that uptake efficiency generally declines with increasing chemical hydrophobicity, which may be caused by a combination of slow kinetics and a short residency time in the gut. Additionally, because the role of metabolism often is not addressed, the apparent uptake efficiency may also result from more effective elimination of these compounds. To avoid confounding the estimate of assimilation, the parent compound plus metabolites must be determined. Additional research that includes uptake and elimination kinetics is needed to better assess uptake efficiency of PAHs for the different routes of uptake, especially the dietary route. These data will help greatly in predicting bioaccumulation from different environmental matrices.

3. Uptake Kinetics. Determination of the rate of uptake can provide very useful information about bioaccumulation. In dynamic environments, where conditions are changing and water–sediment equilibrium is not achieved, a compound that is slowly taken up may allow the animal to avoid harm as it moves between high and low exposure concentrations. The rate of uptake is also of interest under spill conditions where a substance such as oil is released into the environment and achieves high concentrations for a short time. Although it is well known that fish and crustaceans can metabolize PAHs much more efficiently than clams or polychaetes, and hence possess faster elimination rates, it is also apparent that crustaceans and fish have very rapid rates of uptake (Neff et al. 1976), which may be related in part to high ventilatory rates.

The rates of uptake (k_1) within a species under constant conditions have been shown to vary by only a factor of 2 or 3 for PAHs of widely varying hydrophobicity (Bender et al. 1988, Landrum 1988); however, the uptake transfer coefficients (similar to k_1) for naphthalene (0.011 hr^{-1}) and chrysene (0.0015 hr^{-1}) differed by 7.5 times in a deposit-feeding clam (*Macoma balthica*) (Foster et al. 1987). This uptake coefficient was determined for animals exposed to sediment-associated PAHs; hence, some of the difference may be caused by reduced bioavailability of the more hydrophobic PAHs.

While the intraspecific rates of uptake have been shown to be similar for PAHs, the interspecific rates can be very different, even between related species. In a comparative study of short-term uptake, oysters (*Crassostrea virginica*) took up about 3–4 times more than clams (*Rangia cuneata*) of

each of the 13 PAHs measured (Neff et al. 1976). This disparity was probably caused by differences in ventilatory rate, which can control the rate of uptake, and possibly uptake efficiency. Interestingly, for many of the PAHs measured (especially the methylnaphthalenes), the clam had higher rates of elimination than the oyster. Conversely, when this same oyster species was compared to another clam (*Mercenaria mercenaria*), only small differences in the uptake constant were observed for PAHs ranging two orders of magnitude in hydrophobicity (Bender et al. 1988). In another comparative study, Harris et al. (1977) found that the copepod (*Eurytemora affinis*) accumulated naphthalene concentrations that were 50-fold higher than those observed in the copepod (*Calanus helgolandicus*) when based on dry weight (34-fold when based on lipid-normalized concentrations). While a 3-fold higher elimination rate in *C. helgolandicus* may explain some of the differences, individuals of this species weigh, on the average, 17.5 times more than the smaller *E. affinis*. It appears that there is a strong relationship between organism size and accumulation, mainly the rate of uptake (Landrum 1988), although size may not always explain large differences in bioaccumulation (Meador et al. 1993).

Temperature is one factor that can have a dramatic effect on uptake rate. Kennedy et al. (1989) found a high correlation between the Q_{10} value (the factor reflecting a change in a process for a 10 °C change) for ventilatory rate and benzo[a]pyrene uptake in gulf toadfish (*Opsanus beta*). These results indicate that as temperature increased the ventilation rate also increased, which resulted in more benzo[a]pyrene taken up by the fish. They also observed that the uptake rate of benzo[a]pyrene was lower for fish acclimated to 28 °C and exposed at 18 °C than those acclimated and exposed to 18 °C (Fig. 7), suggesting a compensatory mechanism. In contrast, no difference in uptake of dimethylnaphthalene by the grass shrimp (*Palaemonetes pugio*) was observed when exposed at a stable temperature (20 °C) versus a fluctuating temperature (18–22 °C) (Dillon 1982), which was also the case for uptake of anthracene by the clam, (*Rangia cuneata*) when exposed at 10 °, 20 °, and 30 °C (Jovanovich and Marion 1987). This was also confirmed by Bender et al. (1988), who found no temperature effect on the rate of uptake of PAHs for clams (*Mercenaria mercenaria*) or oysters (*Crassostrea virginica*) when exposed at 15 ° or 25 °C to field-contaminated sediment. Paradoxically, Fucik and Neff (1977) reported that even though clams (*Rangia cuneata*) had 2–3 times the filtration rate at 30 °C versus 15 °C, they still accumulated approximately twice as much naphthalene at the lower temperature when compared to the higher temperature. These results are counterintuitive and may be related to increased metabolism and/or excretion at the higher temperature.

B. Elimination

Metabolism, excretion, and diffusive loss, collectively termed *elimination*, are processes that can decrease tissue concentrations of parent PAHs. Studies with fish show that parent LPAHs can be eliminated through the gills,

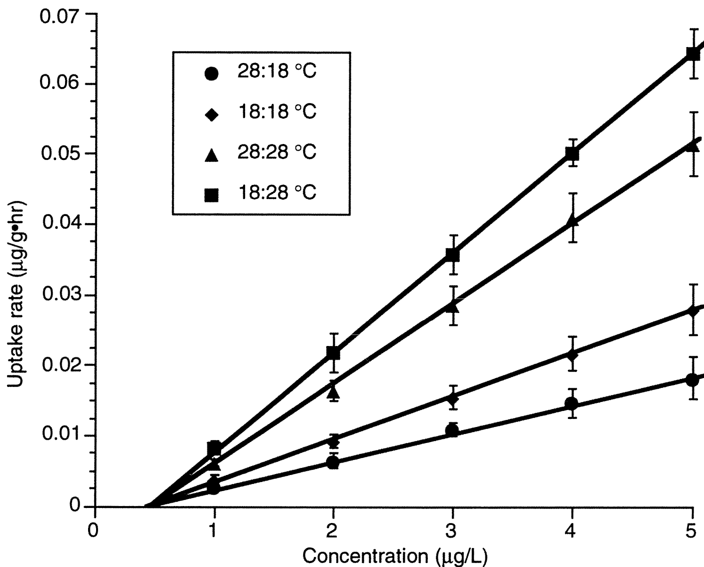

Fig. 7. Rate of uptake of benzo[a]pyrene by the Gulf toadfish (*Opsanus beta*) in relation to water concentration. The first and second numbers refer to acclimation and exposure temperature, respectively. Fish were acclimated for 4 weeks and exposed to benzo[a]pyrene for 24 hr. Uptake rates were calculated as the first derivative of the fitted exponential curve at each sampling time with respect to decreasing chemical concentration. Values are means and standard error of the mean for six fish. (Redrawn from Kennedy et al. 1989.)

whereas HPAHs are not appreciably released by this route (Thomas and Rice 1982). This may also be true for invertebrates; however, we are unaware of studies that have examined this directly. Metabolism will biotransform these xenobiotic parent PAHs to metabolites that are rendered more water soluble to facilitate excretion. Excretion is an active (vis-á-vis passive) physiological process that eliminates parent compounds and metabolites through bile or urine. The term *diffusive loss* is used here to mean a decrease in tissue burden caused by simple diffusion out of the organism that is controlled by the thermodynamic partitioning between compartments (e.g., tissue and water). Metabolism, excretion, and diffusive loss are described as elimination and can be quantitatively described by the elimination rate constant (k_2). We recommend that the word *depuration* be used for the mechanism of diffusive loss and the word *elimination* reserved for the combined processes of metabolism, excretion, and diffusive loss of contaminants (see Baron et al. 1990).

Metabolism of parent compounds is the principal confounding factor in assessing exposure to PAHs. Thus, the measurement of parent hydrocarbons in any organism that is actively metabolizing PAHs will yield only

partial information about the total PAH-derived body burden. Consequently, direct measurement of the parent hydrocarbon would be a useful quantitative tool only in animals that have minimal capacity for PAH metabolism. Both the level of cytochrome P450 (CYP1A in fish) activity and the degree of induction will affect the levels of parent PAHs measured in an organism. Metabolism of PAHs generally occurs via the mixed function oxidase system (MFO), which requires cytochrome P450 and NADPH (reduced nicotinamide adenine dinucleotide phosphate). Some species of invertebrates are capable of metabolizing PAHs via NADPH-independent pathways, which are not mechanistically well understood (Livingstone 1994).

PAHs, unlike PCBs, are readily and often extensively metabolized and frequently leave only a trace of parent compound in tissues of some species, such as fish and marine mammals. In comparison, crustaceans and some polychaetes have a reduced capacity for PAH metabolism. Molluscs have a very limited ability to metabolize PAHs and hence reflect environmental exposure more accurately than the species that can facilely convert parent PAHs to metabolites. Even within major taxa (e.g., crustaceans, polychaetes), a range in ability to biotransform PAHs exists. Thus, it is essential that a species' metabolic capability be assessed before it is utilized in studies of bioaccumulation of PAHs.

Although photodegradation of PAHs as an elimination pathway in marine organisms is potentially very important, it is not discussed here primarily because there are few reports addressing this subject.

1. Elimination in Invertebrates. In marine invertebrates, the ability to metabolize PAHs varies widely within and between phyla, but generally at rates much less than those observed in vertebrates (Livingstone 1994). Lemaire et al. (1993) demonstrated that *in vitro* NADPH-dependent metabolism of benzo[a]pyrene in the liver of flounder (*Platichthys flesus*) was approximately 27 times the rates observed in the digestive glands of the mussel (*Mytilus edulis*) and in the hepatopancreas of the crab (*Carcinus maenas*). The authors noted that NADPH- or NADH-independent metabolism of benzo[a]pyrene also occurred at a relatively low rate in the mussel. For a comprehensive review of xenobiotic metabolism in invertebrates, see the reviews by Livingstone (1992, 1994).

It was once thought that many bivalves lack the enzyme systems needed to biotransform PAHs (Lee et al. 1972; Vandermeulen and Penrose 1978); however, recent studies show relatively short half-lives (days) for many PAHs (Lake et al. 1985; McLeese and Burridge 1987). An early study by Lee et al. (1972) found that mussels (*Mytilus edulis*) were able to accumulate, but not metabolize, radiolabeled naphthalene and benzo[a]pyrene. Another study with mussels (*Mytilus edulis*), clams (*Mya arenaria*), and oysters (*Ostrea edulis*) found no evidence of aryl hydrocarbon hydroxlyase, even after 6 yr of exposure to oil from a shipwreck (Vandermeulen and

Penrose 1978). More recent studies have shown that *Mytilus edulis* has the capacity to metabolize PAHs and aromatic amines (Marsh et al. 1992) in addition to its ability to metabolize benzo[a]pyrene in the absence of NADH or NADPH cofactors (Lemaire et al. 1993). It has also been demonstrated that a *Mytilus* sp. can form reactive metabolites of benzo[a]pyrene that attach to DNA (Marsh et al. 1992).

Studies with polychaetes show a range in biotransformation capability. For example, Kane-Driscoll and McElroy (in manuscript) found that polychaetes from three different families exhibited very diverse abilities to metabolize benzo[a]pyrene. Their study showed that *Nereis divisicolor* (Nereidae) exhibited a biotransformation capacity that was almost an order of magnitude higher than that found in *Leitoscoloplos fragilis* (Orbiniidae), which biotransformed very little of the compound. Another species (*Scolecolepides viridis*; Spionidae) examined in this study exhibited an intermediate response. McElroy (1985) found that the total benz[a]anthracene in a polychaete (*Nereis virens*) was present principally as metabolites (77% metabolized and 23% parent compound) after 6 d of exposure to contaminated sediment. A study with the ophelid polychaete (*Armandia brevis*) found only 35% of the total ^{14}C-benzo[a]pyrene activity in the tissues was from the parent compound after 10 d exposure to spiked sediment (Casillas et al. in manuscript) indicating active metabolism for this polychaete species. Experiments with the lugworm (*Abarenicola pacifica*) found that after 68 d of exposure, only 30% of the total benzo[a]pyrene-derived radioactivity in its tissue was from degradation products, indicating weak biotransformation by this species (Weston 1990). Conversely, Augenfeld et al. (1982) found no metabolites in this species after 60 d of exposure, indicating either a lack of enzymatic capability or a very long lead time for induction of less specific enzymatic pathways.

Crustaceans also exhibit a range in ability to biotransform PAHs. A study with the blue crab (*Callinectes sapidus*) found that this species was able to extensively metabolize PAHs (naphthalenes, fluorene, and benzo[a]pyrene) and produce high concentrations of metabolites (Lee et al. 1976). Conversely, Burns (1976) found that another crab (*Uca pugnax*) possessed a very weak, uninducible microsomal MFO system for metabolizing xenobiotic hydrocarbons. It was noted that this species was very sensitive to oil pollution because of its limited ability to metabolize hydrocarbons. The larval spot shrimp (*Pandalus platyceros*) only weakly metabolized naphthalene with 9%–21% of the total ^{14}C-naphthalene in tissues present as metabolites (Sanborn and Malins 1977). Contrary to this, fairly extensive biotransformation of naphthalene was demonstrated for the copepod *Calanus helgolandicus* by Corner et al. (1976) and Harris et al. (1977).

Some studies indicate that species in related taxonomic groups can have different metabolic capacities and that any assumptions based on results from similar species can lead to erroneous conclusions. A comparative study of amphipods [*Rhepoxynius abronius* (Phoxocephalidae) and *Eo-*

haustorius washingtonianus (Haustoriidae)] from the superfamily Haustorioidea exposed to sediment spiked with ^{14}C-benzo[a]pyrene concentrations found that the parent compound was 2.6 times higher in *E. washingtonianus*, while total soluble benzo[a]pyrene metabolite concentrations were 2.3 times higher in *R. abronius* (Reichert et al. 1985). While a twofold difference in metabolism may not seem large, the higher rate of metabolism will lead to more reactive metabolites being formed, which should result in increased levels of binding to cellular macromolecules, including DNA. The results of Reichert et al. (1985) showing that *R. abronius* contained significantly higher levels of benzo[a]pyrene metabolites covalently bound to macromolecules supports this hypothesis. The high rate of PAH metabolism in *R. abronius* was corroborated by Casillas et al. (in manuscript) who showed that only 15% of the total ^{14}C-benzo[a]pyrene was present as parent compound after 10 d exposure to sediment spiked with this compound. Another interesting comparison can be made for two macruran decapod crustaceans (*Homarus americanus* and *Panulirus argus*). The half-life for ^{14}C-benzo[a]pyrene in *P. argus* was 1–2 wk (Little et al. 1985), whereas 6.5 wk following a single injection of ^{14}C-benzo[a]pyrene into *H. americanus*, 80%–90% of the total radiolabel remaining was found in the hepatopancreas and 80% of this was present as parent compound, indicating a weak rate of biotransformation (Bend et al. 1981; Foureman et al. 1978). Recognition of these potential differences will help in the proper interpretation of PAH body burdens in organisms and extrapolation to other species.

2. Elimination in Fish. PAHs are rapidly taken up by fish and metabolized, and the metabolites are generally excreted into bile, which is a major route of elimination (Varanasi et al. 1989a). In fish, metabolism can rapidly convert up to 99% of the PAH compounds to metabolites within 24 hr of uptake that markedly reduces tissue concentrations of parent hydrocarbon (Varanasi et al. 1989a). For example, studies with radiolabeled benzo[a]pyrene in English sole (*Parophrys vetulus*) show that in liver the proportion of parent compound is less than 3% of the total compound present (parent plus metabolites) (Varanasi et al. 1989a). In addition to the many factors just mentioned that can lead to differences in the rate of elimination within a species, biotransformation and excretion rates can vary widely between fish species. The mechanisms of PAH elimination in fish have been recently reviewed by Varanasi et al. (1989a).

3. Elimination Kinetics. Determination of the rate of elimination is a useful exercise that can be used to calculate the half-life ($t_{1/2}$) and determine the persistence of PAHs in tissue, in addition to modeling steady-state tissue burdens. The balance between uptake and elimination will determine the bioconcentration or bioaccumulation factor, which can be compared to an expected value (for example, see BCF_{pred} in Appendix). Computation of half-life is also a good benchmark for interspecific comparison of the per-

sistence of PAHs in tissue. The half-life of a contaminant chemical in tissue is usually expressed in terms of hours or days, or more usefully, in terms of the elimination rate (k_2). Several half-lives for various species and PAHs are listed in Table 2, and a review of half-lives of chemicals in fish can be found in Niimi (1987).

Because PAHs exhibit a range in lipophilic affinity, elimination that relies solely on passive diffusion loss should be slower for the more hydrophobic PAHs. Such a correlation has been shown for the freshwater amphipod [*Diporeia* spp. (*Pontoporeia hoyi*)], which does not metabolize PAHs to any appreciable extent (Landrum 1988). This author found that for a series of PAHs with increasing octanol–water partition coefficients, the k_2 elimination constant decreased, which led to longer half-lives. The more water-soluble PAHs phenanthrene, anthracene, and fluoranthene showed nearly identical rapid elimination patterns ($t_{1/2} \approx 2$ d) in mussels (*Mytilus edulis*) exposed to contaminated suspended sediments for 28 d, while the more hydrophobic PAHs produced half-lives in the range of 4–6 d (Fig. 8; see Table 2) (Lake et al. 1985). The most hydrophobic of the group, perylene, displayed the slowest elimination.

We have plotted the k_2 constants from Bender et al. (1988), a comparative study of elimination between a clam (*Mercenaria mercenaria*) and an oyster (*Crassostrea virginica*), in order of increasing PAH hydrophobicity along the abscissa of Fig. 9. Benzo[ghi]fluoranthene was excluded because no K_{ow} was found and perylene because it is a naturally occurring PAH. Interestingly, the oyster exhibited declining k_2 elimination constants as hydrophobicity increased, which may be indicative of little or no metabolism; however, the clam appears to have essentially the same rate of elimination across all PAHs, which may indicate active metabolism. Table 2 shows that the half-lives for PAHs in this clam species are all relatively short compared to those in the oyster. This observation would suggest that diffusive loss, as controlled by chemical nydrophobicity, will determine the minimum k_2 value and any biotransformation activity will only increase the rate of elimination. In cases where biotransformation is substantial, the pattern of a decreasing rate of elimination with increasing hydrophobicity may be masked.

It is unclear why a different elimination rate should occur in animals depending on whether they take up PAHs through the gills or the gut. When adult female copepods (*Calanus helgolandicus*) accumulated naphthalene through the diet, their elimination rate was approximately 20% of that for water exposure (Corner et al. 1976). After 10 d in clean water, about 30% of the naphthalene from dietary uptake remained in the tissues while only 5% from water uptake remained. These results suggest that with dietary uptake the PAH is distributed to compartments from which elimination is less rapid.

Temperature and season may also affect the rate of elimination of PAHs from tissue. Jovanovich and Marion (1987) found large differences in the elimination of anthracene by the clam *Rangia cuneata* when exposed at 10,

Table 2. Half-life of polycyclic aromatic hydrocarbons (PAHs) in marine organisms.

Species	PAH	$t_{½}$ (d)	Exp	Ref
Mussel1	benzo(a)pyrene	16	6	1
Oyster	benz(a)anthracene	7	1	2
Oyster	naphthalenes	2	1	2
Rainbow trout	fluorene	7	3	3
Rainbow trout	phenanthrene	9	3	3
Rainbow trout	anthracene	7	3	3
Rainbow trout	fluoranthene	6	3	3
Rainbow trout	phenyl naphthalene	25	3	4
Rainbow trout	acenaphthylene	1	3	4
Rainbow trout	2-methyl anthracene	2	3	4
Rainbow trout	triphenylene	2	3	4
Rainbow trout	9-methyl anthracene	4	3	4
Rainbow trout	perylene	2	3	4
Mussel1	fluoranthene	29.8	5	5
Mussel1	benz(a)anthracene	17.8	5	5
Mussel1	chrysene	14.2	5	5
Mussel1	benzo[b]fluoranthene	16.9	5	5
Mussel1	benzo[k]fluoranthene	11.9	5	5
Mussel1	benzo[e]pyrene	14.4	5	5
Mussel1	benzo[a]pyrene	15.4	5	5
Mussel1	indeno[1,2,3-cd]pyrene	16.2	5	5
Mussel1	benzo[ghi]perylene	15.4	5	5
Clam1	phenanthrene	4.5	1	6
Clam1	fluoranthene	8.4	1	6
Clam1	pyrene	10.3	1	6
Clam1	triphenylene	4.4	1	6
Clam1	perylene	26.2	1	6
Mussel1	phenanthrene	2.2	1	6
Mussel1	fluoranthene	11.1	1	6
Mussel1	pyrene	4.1	1	6
Mussel1	triphenylene	8.0	1	6
Mussel1	perylene	13.3	1	6
Polychaete2	benzo[a]pyrene	7	4	7
Mussel1	phenanthrene	1.9	5	8
Mussel1	anthracene	1.9	5	8
Mussel1	fluoranthene	2.0	5	8
Mussel1	pyrene	5.5	5	8
Mussel1	benz(a)anthracene	4.3	5	8
Mussel1	chrysene	5.0	5	8
Mussel1	benzo[b]fluoranthene	5.7	5	8
Mussel1	benzo(e)pyrene	6.9	5	8
Mussel1	benzo(a)pyrene	4.8	5	8
Mussel1	perylene	6.3	5	8
Clam4	naphthalene	2.0	4	9
Clam4	chrysene	3.3	4	9
Oyster	phenanthrene	3.4	2	10
Oyster	pyrene	6.7	2	10

(*Continued*)

Table 2. (*Continued*)

Species	PAH	$t_{½}$ (d)	Exp	Ref
Oyster	1-methlphenanthrene	6.7	2	10
Oyster	fluoranthene	5.9	2	10
Oyster	methylpyrene	10.5	2	10
Oyster	benzo[a]fluorene	10.5	2	10
Oyster	benzo[b]fluorene	9.6	2	10
Oyster	chrysene	15.1	2	10
Oyster	benz[a]anthracene	15.4	2	10
Oyster	benzo[e]pyrene	30.1	2	10
Oyster	benzo[a]pyrene	21.7	2	10
Oyster	perylene	9.2	2	10
Oyster	benzo[ghi]fluoranthene	12.4	2	10
Oyster	benzofluoranthene	77.0	2	10
Clam2	phenanthrene	6.1	2	10
Clam2	pyrene	3.6	2	10
Clam2	1-methylphenanthrene	6.0	2	10
Clam2	fluoranthene	3.3	2	10
Shrimp	phenanthrene	0.9	1	6
Shrimp	fluoranthene	0.8	1	6
Shrimp	pyrene	0.8	1	6
Shrimp	triphenylene	2.4	1	6
Shrimp	perylene	1.2	1	6
Polychaete1	phenanthrene	4.8	1	6
Polychaete1	fluoranthene	5.8	1	6
Polychaete1	pyrene	4.8	1	6
Polychaete1	triphenylene	14.4	1	6
Polychaete1	perylene	5.7	1	6
Clam2	benzo[a]fluorene	4.2	2	10
Clam2	benzo[b]fluorene	4.3	2	10
Clam2	chrysene	4.3	2	10
Clam2	benz[a]anthracene	4.0	2	10
Clam2	benzo[e]pyrene	4.7	2	10
Clam2	benzo[a]pyrene	8.0	2	10
Clam2	perylene	4.3	2	10
Clam2	benzo[ghi]fluoranthene	4.8	2	10
Clam2	benzofluoranthene	3.9	2	10
Clam3	benzo[a]pyrene	8.0	1	11

Mussel1 (*Mytilus edulis*), mussel2 (*Mytilus californianus*), oyster (*Crassotrea virginica*), rainbow trout (*Oncorhynchus mykiss*), clam1 (*Mya arenaria*), shrimp (*Crangon septemspinosa*), polychaete1 (*Nereis virens*), polychaete2 (*Abarenicola pacifica*), clam2 (*Mercenaria mercenaria*), clam3 (*Rangia cuneata*), clam4 (*Macoma balthica*).

Exp is exposure conditions: 1, lab exposure with water; 2, lab exposure of field-contaminated sediment; 3, food spiked with compound; 4, lab exposure of labeled (spiked) sediment; 5, lab exposure of field-contaminated suspended sediment; 6, field-contaminated animals held in clean lab water. The formula for half-life ($t_{½}$) = $0.693/k_2$ was used to convert some k_2 values to half-lives.

Ref is reference; included are time of exposure and temperature for elimination (days, temp °C): 1, Dunn and Stich 1976a (chronic, 8 °C); 2, Lee 1977 (9 d, –); 3, Niimi and Palazzo

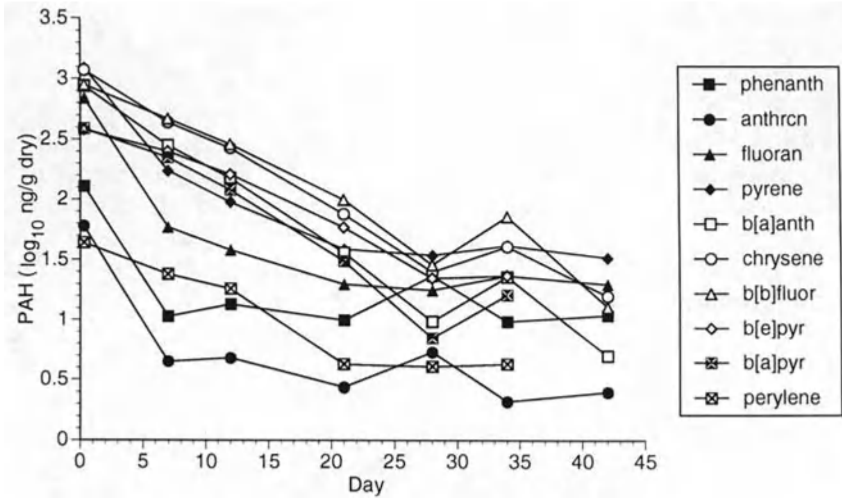

Fig. 8. Elimination of PAHs by the mussel *Mytilus edulis*. Mussels were exposed for 28 d to field-contaminated suspended sediment in the laboratory and then allowed to eliminate PAHs from tissues. See Appendix for chemical abbreviations. (Data from Lake et al. 1985.)

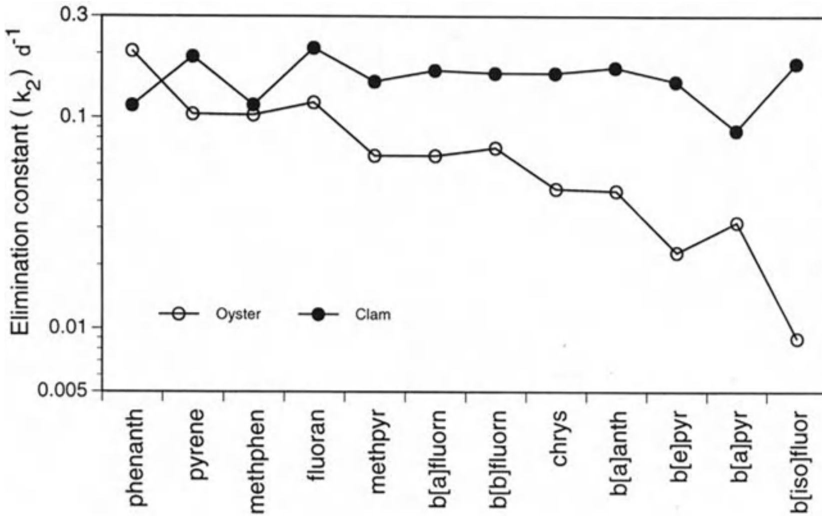

Fig. 9. Polycyclic aromatic hydrocarbon elimination constants for oyster (*Crassostrea virginica*) and clam (*Mercenaria mercenaria*). Bivalves were exposed for 28 d to field-contaminated sediment and then allowed to eliminate their acquired tissue concentrations for 28 d. See Appendix for chemical abbreviations. (Data from Bender et al. 1988.)

(1986) (1 d, 11 °C); 4, Niimi and Dookhran (1989) (5 d, 15 °C); 5, Pruell et al. (1986) (40 d, 15 °C); 6, McLeese and Burridge (1987) (4 d, 10 °C); 7, Weston (1990) (50 d, 10 °C): 8, Lake et al. 1985 (28 d, 15 °C); 9, Foster et al. (1987) (12 d, 21 °C); 10, Bender et al. (1988) (28 d, 25 °C); 11, Neff et al. 1976 (1 d, –), –, data not available.

20, and 30 °C at different times of the year. They found that elimination was generally fastest during the fall and winter. Half-lives ranged fivefold (6.1–31.5 d) over the year at 10 °C and about 2.5-fold at 20 °C (4.9–12.6 d) and 30 °C (3.8–9.5 d) and appear to be related to the reproductive cycle, with high elimination during spawning and much slower elimination during gametogenesis. When comparing temperature only, the rate of elimination of anthracene was always slower at 10 °C compared to 20 ° and 30 °C. Conversely, Fucik and Neff (1977) found no effect of temperature or salinity on the release of naphthalenes from clams (*Rangia cuneata* and *Protothaca staminea*). Additional descriptive studies are needed to assess the effects of temperature on PAH elimination, especially as it relates to reproductive state.

4. Half-Life of PAHs. Because some invertebrates are capable of rapidly metabolizing PAHs and others have limited ability to biotransform these compounds, a large range in half-lives of PAHs in marine organisms has been reported (see Table 2). Some studies report rapid half-lives on the order of hours, but most report half-lives on the order of a few days to a week or more. Some studies report a classic fast and slow (biphasic) component to elimination. The fast component of elimination may indicate a half-life of a few days to a week; however, the slow component is an asymptotic elimination of some PAHs that may indicate long-term retention of a significant proportion of accumulated xenobiotics.

A number of short-term exposure experiments have shown rapid half-lives for PAHs. For example, Neff et al. (1976) found that clams (*Rangia cuneata*) that had accumulated 7.2 μg/g of benzo[a]pyrene over 24 hr lost 60% of this compound after 10 d in clean seawater. Clams (*Tapes japonica*) exposed for a few days to several PAHs exhibited half-lives that were relatively short (4–8 d) (Obana et al. 1983). Fast elimination rate constants (k_2) were measured in a clam (*Macoma balthica*), and only a 1.6-fold difference was detected between naphthalene (0.0141 hr^{-1}; $t_{½}$ = 49.1 hr) and chrysene (0.0087 hr^{-1}; $t_{½}$ = 79.7 hr) (Foster et al. 1987).

Conversely, there are studies that report longer half-lives for PAHs. Mussels (*Mytilus edulis*) that had been naturally exposed to benzo[a]pyrene from creosote-treated timbers were placed in clean seawater; after 42 d of elimination the tissue concentrations dropped from 45 ng/g (wet weight) to less than 10 ng/g, producing a half-life of 16 d (Dunn and Stich 1976a). However, mussels maintained out of water for 3 d showed no decrease in tissue burden (the submerged group lost 20%), which may be a function of anaerobic metabolism. These authors concluded that mussels could not metabolize benzo[a]pyrene but they could reduce tissue concentrations through diffusive loss, albeit slowly. Other studies have also reported long half-lives for PAHs in chronically exposed animals. For example, Pruell et al. (1986) found a half-life of 17.8 d for benz[a]anthracene and 29.8 d for fluoranthene in uncontaminated mussels (*Mytilus edulis*) exposed to field-contaminated sediment for 40 d and then placed in clean seawater.

Although the passive release or metabolism of PAHs may be slower in animals that were chronically exposed, the half-life may also depend on the hydrophobicity of the PAH considered (Spacie and Hamelink 1982). Neff et al. (1976) reported that the rate of release of a PAH is dependent on molecular weight, and presumably hydrophobicity, with the higher-molecular-weight compounds being released much more slowly than low-molecular-weight compounds. They found a relatively slow release of benzo[a]pyrene compared to naphthalenes, with 29% of the benzo[a]pyrene still present in tissue after 10 d in clean seawater.

In fish, the half-lives of PAHs are generally very short. Half-lives of 6–9 days for fluorene, phenanthrene, anthracene, and fluoranthene were reported in rainbow trout [*Oncorhynchus mykiss* (*Salmo gairdneri*)] fed oil spiked with PAHs (Niimi and Palazzo 1986). Niimi and Dookhran (1989) also reported half-lives of 1–4 d for acenaphthylene, methylanthracene, triphenylene, and perylene in rainbow trout. They reported a much longer half-life (25 d) for phenylnaphthalene compared to other PAHs and speculated that it may be more recalcitrant to metabolism because its aromatic rings are not all fused.

Consideration of enzyme kinetics and diffusion rates may help in understanding the patterns of PAH half-lives in marine organisms. A study by Schnell et al. (1980) on the kinetics of metabolism of PAHs by monooxygenases in salmon (*Oncorhynchus kisutch*) liver microsomes found widely varying Michaelis constants (K_m) for three PAHs. The K_ms for naphthalene, 2,6-dimethylnaphthalene, and benzo[a]pyrene were 300 $\mu\underline{M}$, 15.3 $\mu\underline{M}$, and 2.1 $\mu\underline{M}$, respectively, indicating a two-order-of-magnitude range in affinity of the enzyme for PAH substrates. At tissue concentrations much below enzyme saturation ($V_{max} \geq 80\ \mu\underline{M} \approx 20\ \mu g/g$) in this species, the lower K_m for benzo[a]pyrene would indicate a turnover rate that was more than 100 times faster than that observed for naphthalene. Because we observe only small differences in half-life between PAHs such as naphthalene and benzo[a]pyrene in those species that actively metabolize xenobiotic compounds (see Table 2), other factors must be important. One consideration is that HPAHs will slowly diffuse out of lipid-rich storage tissues, which limits the amount of PAH available for metabolism. Additionally, an LPAH such as naphthalene can diffuse easily out of a fish through its gills, whereas an HPAH is not appreciably eliminated by this route (Thomas and Rice 1982). Therefore, these processes suggest that a combination of fast diffusion for the LPAHs balanced by fast metabolism of the HPAHs can lead to relatively small differences in PAH half-life as hydrophobicity increases. These types of studies are rare, and additional effort in this area will help greatly in elucidating the actual mechanisms of PAH elimination.

5. Persistence in Marine Organisms. Recent research suggests that bioaccumulation has an acute and chronic phase that may affect the rate of elimination and persistence of PAHs in tissue. It has been suspected that the time of exposure determines the persistence of a given PAH, even in

species with high MFO activity. In general, there seems to be agreement that the LPAHs are less persistent than the HPAHs, as has been discussed. One mechanism for this may be that both LPAHs and HPAHs in tissues are controlled by simple diffusion in and out of lipid pools and become less available for metabolic transformation once sequestered by fatty tissue. These lipid-rich tissues are generally less well perfused, which leads to reduced mobilization of the compounds. Based on simple diffusion kinetics, HPAHs will diffuse more slowly out of an organism compared to LPAHs and therefore would be more persistent. Short-term exposure may not allow the hydrophobic HPAHs to partition into these lipid pools; hence, in the short term they are more labile and easily eliminated.

Those species possessing low MFO activity will generally have high persistence of parent PAHs in their tissues and will more closely reflect environmental exposure. This area of research has received much attention because it is desirable to know if individuals can accumulate high levels of PAHs from spill events and whether these PAHs are retained or quickly return to background levels after the acute event has passed. Obviously some of the contradictions among studies occur because the capacity for biotransformation has not been fully determined; however, there is conflicting information regarding persistence in tissues, even for a given PAH in one species.

Accumulation of PAHs over a short period of time appears to allow rapid elimination, especially for LPAHs, when the animal is placed in water of reduced concentration. For example, mussels (*Mytilus edulis*) exposed during the first 5 d of an oil spill accumulated high tissue concentrations of hydrocarbons, but after 1 wk in cleaner waters were able to return to background levels (DiSalvo et al. 1975). These authors also found that mussels (*Mytilus californianus*) from a reference area exposed to San Francisco Bay water for 3 mon rapidly accumulated hydrocarbons and were able to eliminate 90% of their acquired burden of aromatic hydrocarbons (≈ 80 μg/g dry wt) after 5 wk in clean water.

Other studies found persistent PAHs in organisms from chronically polluted environments. For example, mussels transplanted from a chronically polluted bay to clean waters retained a large percentage of their hydrocarbon load over a 10-wk period (DiSalvo et al. 1975). Chronically exposed clams (*Mercenaria mercenaria*) lost only 23% of their total aromatic hydrocarbons after 97 d of exposure in clean seawater (Boehm and Quinn 1977), and a persistent fraction of accumulated hydrocarbons found in oysters (*Crassostrea virginica*) was probably due to sequestering of PAHs in a stable compartment within the organism (Stegeman and Teal 1973). There is evidence that in some cases a rapid, first-order elimination occurs initially, followed by a much slower rate of release that is a function of the time of exposure. For example, fast and slow elimination of naphthalene, which varied by more than 100-fold, was detected between tissues within mussels (*Mytilus edulis*) (Widdows et al. 1983).

Many studies have discovered that animals exposed to naphthalene rapidly eliminate this compound once exposed to uncontaminated conditions. For example, Lyes (1979) found that lugworms (*Arenicola marina*) exposed to ^{14}C-naphthalene seawater for a short period of time (12 hr) rapidly lost this compound from tissue and were no different from control animals after 24 hr. Even with a longer exposure time, Anderson et al. (1977) found that the naphthalene that had accumulated in a sipunculid worm (*Phascolosoma agassizii)* over 2 wk of exposure was rapidly lost when individuals were transferred to clean sediment. Additional long-term studies with an LPAH such as naphthalene will help determine if PAHs with K_{ow}s in this range may be persistent.

One study of PAH elimination with lobsters (*Homarus americanus*) showed an interesting pattern (Uthe and Musial 1986). Lobsters that were captured from a polluted area of Sydney Harbor, Nova Scotia, Canada and held for 12 mon in a relatively clean aquarium system retained a large percentage of some of the PAHs in their tissues. The results showed a relationship between the percentage decline and PAH water solubility (an index of hydrophobicity) (Fig. 10). For the more water-soluble PAHs (e.g., fluoranthene and pyrene), the lobsters lost about 80% of their initial body burden, whereas for the more hydrophobic compounds (e.g., benzo[a]py-

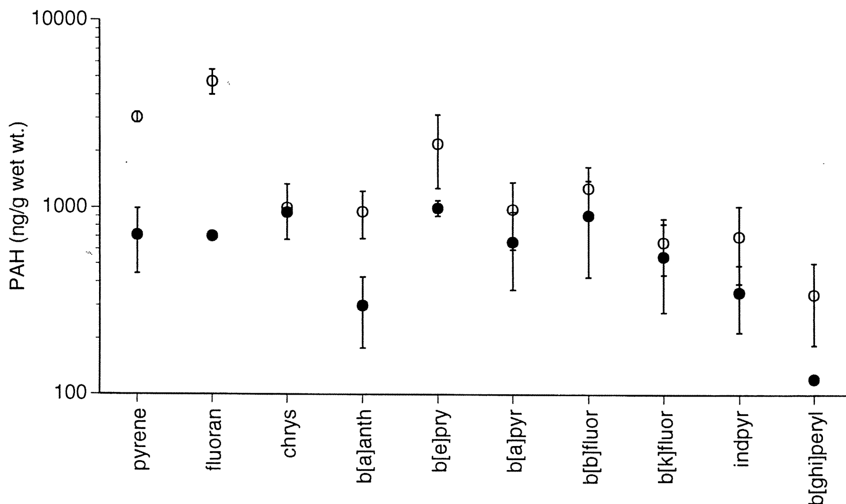

Fig. 10. Polycyclic aromatic hydrocarbons in lobster (*Homarus americanus*). The concentrations of several PAHs (ordered by increasing K_{ow}) were determined in the hepatopancreas at the time of capture (*open symbols*) from Sydney Harbour, Nova Scotia, and again after holding for 12 mon in the laboratory (*closed symbols*). Values are mean and standard deviation. See Appendix for chemical abbreviations. (Data from Uthe and Musial 1986.)

rene, benzo[ghi]perylene) most to all of the PAH was retained in the tissues. Similar results were found for both digestive gland and tail muscle. This observation is probably related to the slow rate of biotransformation found in this species for PAHs that was discussed earlier.

One advantage of this rapid release of aromatic hydrocarbons accumulated from acute exposure would apply to exposure from spills and other acute events. If the event is only a few days in duration, it is highly probable that most of the accumulated aromatic hydrocarbons would be eliminated and or metabolized to low levels, depending on the species. For example, tissue concentrations of anthracene, fluoranthene, benzo[a]pyrene, and benz[a]anthracene in zooplankton (primarily *Pseudocalanus minutus*) were not persistent and declined to below detection concentrations (0.1 μg/g) during the 9 d after a pulse of oil was added to a mesocosm (Lee et al. 1978). Conversely, the chronic exposure seems to produce tissue burdens that are relatively stable, especially for the more hydrophobic of the PAHs.

These results indicate that after a short-term exposure organisms may eliminate almost all of their acquired burden of parent compound, whereas during chronic exposure, an appreciable fraction of the PAH may become incorporated into storage lipids and become less available for diffusive loss or metabolism. This stability is useful in monitoring programs that attempt to use organisms as integrators of contamination.

Even though organisms that accumulate high body burdens of PAHs may apparently eliminate these compounds, the PAHs still may be present in tissues but in another form. One study with an invertebrate showed that when crabs (*Callinectes sapidus*) accumulated high levels of benzo[a]pyrene, concentrations of the parent compound declined almost 90% after 10 d in clean seawater; however, the levels of polar metabolites showed very little reduction, indicating that the compounds had not left the body but were still present (mainly in the hepatopancreas) in another form (Lee et al. 1976).

Parent PAHs and their unbound metabolites are not highly persistent in fish, and the levels remaining in tissues and fluids 2 wk after exposure are generally minimal (Varanasi et al. 1989a); however, reactive PAH metabolites that covalently bind to DNA can be quite persistent in fish. In studies with PAHs and nitrogen-containing aromatics, Stein et al. (1993) have shown that benzo[a]pyrene (a model mutagenic PAH) and 7H-dibenzo-[c,g]carbazole (a model nitrogen containing mutagenic PAH) form highly persistent DNA adducts in fish. In English sole (*Parophrys vetulus*), approximately 40% of the maximum levels of adducts formed were still present 3 mon after exposure. Additional work by Sikka et al. (1991) found that benzo[a]pyrene-DNA adducts persisted for at least 70 d in tissues of brown bullhead (*Ictalurus nebulosus*). Recent work shows that an appreciable proportion of the DNA adducts formed are persistent and that the levels of hepatic DNA adducts formed are dose dependent (Stein et al. 1995a). Additional work has shown that these adducts increase with time of exposure to

PAH-contaminated sediment (French et al. in manuscript). These studies suggest that hepatic DNA adducts, in contrast to parent PAHs, reflect cumulative uptake of PAHs in fish.

6. Time to Steady-State Tissue Burdens. The time required to reach steady-state tissue concentrations in the uptake phase can be determined by measuring the contaminant elimination rate (k_2). The formula (TSS_{50}) = $0.693/k_2$ for time to 50% of steady state, also used to calculate tissue half-life, was used to determine when 50% of the steady-state tissue concentration would be expected if constant uptake is assumed. The time to 95% of steady state can be determined with $2.99/k_2$, or 4.3 times the time to 50% steady state. In Fig. 11, plotted tissue burden versus time to show how k_2 elimination rates affect the time to steady state. The elimination rate defines the shape of these curves and determines when tissue concentration will achieve steady state, because under the conditions of constant uptake and no elimination, tissue concentration would continue to increase indefinitely. The rate of uptake determines the steady state tissue concentration for a given rate of elimination and has no effect on the time to steady state. Many studies give elimination constants that can be used to compute the time to steady state. Table 2, which lists tissue half-lives for various PAH compounds, may also be used to indicate the time needed to achieve 50% of the maximum tissue burden in the uptake phase. The majority of k_2 values reported for PAH elimination in marine organisms occur between

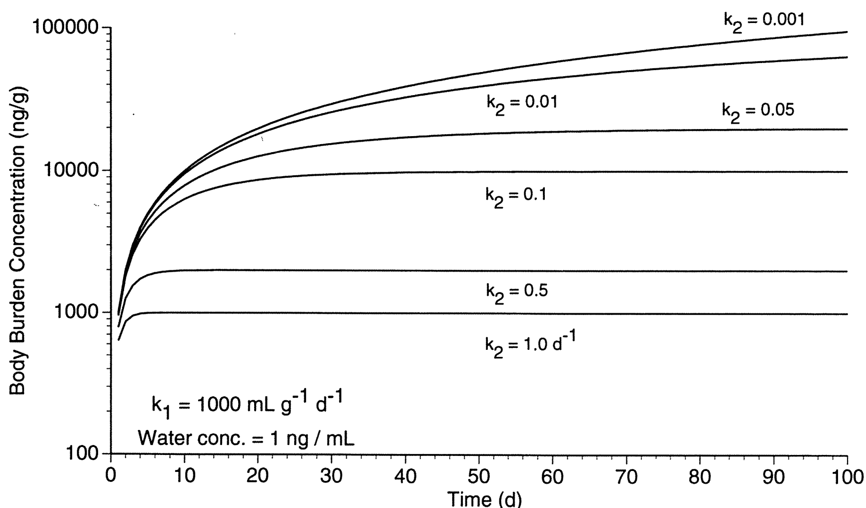

Fig. 11. Model of bioaccumulation over time under constant exposure. Curves were generated with the uptake clearance (k_1) and elimination rate constants (k_2). The formula used for determination of tissue concentration over time is [tissue] = (k_1/k_2)[water]$(1 - e^{-k2t})$.

0.1 and 0.05 d^{-1}, which means that 50% of steady-state tissue concentration generally occurs within 7–14 d. Elimination rate constants for fish generally range from 0.1 to 0.5 d^{-1}.

It is expected that the time to reach steady state is generally species specific and should correlate with chemical hydrophobicity in those animals that eliminate the xenobiotic only through diffusive loss (i.e., cannot biotransform or regulate tissue burden). For those species that can metabolize xenobiotics, the time to steady state can be rapid. Hence, it is possible that steady-state body burdens are achieved slowly in one species and quickly in another due to interspecific differences in the rates of elimination.

The time to attain 50% of steady-state body burdens appears to be relatively fast in several different species. For example, Pruell et al. (1986) found that mussels (*Mytilus edulis*) had accumulated a large portion of their steady-state PAH burden after 10 d when exposed to a sediment slurry containing a PAH profile skewed toward the HPAHs (Fig. 12). Between 10 and 20 d, the tissue burdens had not increased greatly, but between 30 and 40 d tissue burdens started to decline, possibly due to metabolic activation that could have caused reduced concentrations of the parent compounds. Another possibility is that the animals were suffering from toxic effects of PAH accumulation that could reduce their pumping rate and hence reduce their rate of uptake. This theme of rapid attainment of steady-state tissue concentrations has been echoed by many authors. Studies with a deposit-feeding clam (*Macoma balthica*) (Foster et al. 1987), a mussel (*Mytilus edulis*) (Lake et al. 1985; Widdows et al. 1983), a suspension-feeding clam (*Tapes japonica*) (Obana et al. 1983), a deposit-feeding polychaete (*Abarenicola pacifica*) (Augenfeld et al. 1982; Weston 1990), an omnivorous polychaete (*Nereis virens*) (Lake et al. 1985), an oyster (*Crassostrea virginica*) (Pittinger et al. 1985), a filter-feeding clam (*Rangia cuneata*) (Neff et al. 1976), and a deposit-feeding polychaete (*Armandia brevis*) (Meador et al. 1995) all found tissue burdens close to steady state (or were within a factor of 2 or 3) within several days to 2 wk after initiating exposure to both LPAHs and HPAHs.

Some of the observed patterns of accumulation of PAHs from laboratory and field studies may be explained by the rates of uptake and elimination. These rates can be affected by several environmental and physiological factors; hence, care must be taken to define the conditions under which bioaccumulation is observed. A laboratory investigation that compared accumulation of PAHs in oysters (*Crassostrea virginica*) and clams (*Mercenaria mercenaria*) from field-contaminated sediments found that the oyster accumulated about three times as much PAH as the clam (Bender et al. 1988). For most individual PAHs, the ratios between oyster and clam tissue concentrations were between 1 and 5. The authors found no species differences in uptake clearance (k_1) and concluded that the differences in equilibrium BCF values resulted from the higher elimination rate (k_2) in the clam for most PAHs, which would lead to lower body burdens for a given

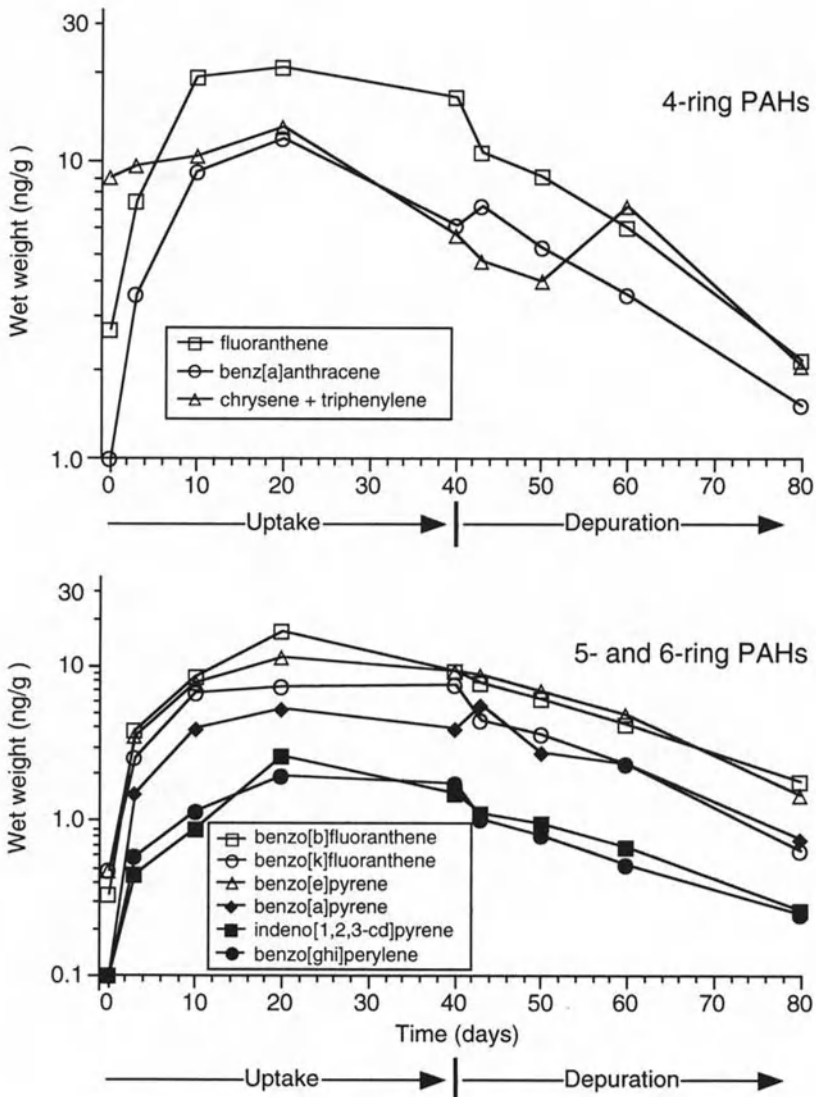

Fig. 12. Tissue concentrations of PAHs in *Mytilus edulis* exposed to field-contaminated suspended sediment from the Providence River, Rhode Island. (Redrawn from Pruell et al. 1986.)

exposure concentration (Fig. 9). Additional research that examines the rates of uptake and elimination in different species over a series of PAHs will add greatly to our knowledge and ability to predict bioaccumulation of PAHs from the environment.

VI. Occurrence in Tissues of Marine Organisms

The occurrence of PAHs in tissue reflects an organism's exposure to contaminated water, sediment, and food, and is a good indication of relative risk. Determination of PAHs in tissue and assessment of exposure is difficult, however, because many organisms can metabolize PAHs, which means they can be exposed, accumulate, and show effects but not contain any measurable amount of the parent contaminant. This is particularly true in fish (Varanasi et al. 1989a), which generally have very low concentrations of parent PAHs in tissues but can have high concentrations of PAH metabolites in bile, indicating uptake of PAHs, metabolism, and excretion. As noted, invertebrates, which have a lesser ability to metabolize these compounds, are more likely to exhibit tissue concentrations of parent compounds that reflect the true exposure. Table 3 presents some of the studies that have measured PAHs in marine organisms with variable metabolic capacity to illustrate the range in concentrations that can be encountered. Total PAHs are included in addition to benzo[a]pyrene, a highly carcinogenic HPAH of potential concern to people who ingest seafood. Table 3 is not exhaustive, but shows that many organisms in diverse locations can accumulate PAHs, frequently to high concentrations. Additional data for marine and freshwater organisms can be found in Neff (1979), Eisler (1987), Wade et al. (1988), and Mearns et al. (1991).

Some species can exhibit high concentrations of PAHs, even in only moderately contaminated areas. For example, some invertebrates (limpets, mussels, periwinkles) from sites in southern Norway exhibited unexpectedly high PAH tissue burdens (Knutzen and Sortland 1982) (Table 3). The sites, which were considered to be relatively pristine to only moderately polluted, ranged from open coastline to small harbors with moderate boat traffic, so it is surprising that high tissue concentrations were found. In many cases the majority of the tissue burdens were composed of LPAHs, which would indicate petroleum sources; however, in several cases HPAHs added significantly to the total PAH body burden. As pointed out by Knutzen and Sortland (1982), benzo[a]pyrene can occur at levels of 5–10 ng/g in marine organisms far removed from the sources of contamination. They also pointed out that even though benzo[a]pyrene is considered highly carcinogenic (Dipple et al. 1984), many other foods such as vegetables, vegetable oil, and smoked and charcoal-broiled foods have benzo[a]pyrene concentrations in the range of 1–10 ng/g (wet weight).

Many surveys over wide geographical areas often report large ranges in PAH concentrations in organisms. For example, Wade et al. (1988) compiled a list of the total PAH concentrations measured in oysters from the Gulf of Mexico and reported values ranging from <20 ng/g to 18.6 μg/g (dry weight). Very high concentrations were found in a survey of lobsters (*Homarus americanus*) from 19 areas in the Canadian maritime provinces, with PAHs ranging from 158 ng/g to 32.6 μg/g (dry weight) in hepatopancreas and below detection to 3.2 μg/g (dry weight) in tail muscle (McLeese 1983).

We found one study that mentioned an impact to commercial fishing caused by elevated PAHs in organisms. In 1982, an area in Sydney Harbor, Nova Scotia, Canada was closed to commercial lobster (*Homarus americanus*) fishing because of high PAH concentrations in the tissues. Individuals sampled in 1982 contained up to 73 $\mu g/g$ (wet weight) in the digestive gland and up to 2.5 $\mu g/g$ total PAHs in tail muscle. By comparison, lobsters from a reference area contained about 0.3 $\mu g/g$ in the digestive gland and about 0.08 $\mu g/g$ (wet weight) total PAHs in the tail muscle (Uthe and Musial 1986).

The rapid metabolism of PAHs in marine fish and some invertebrates precludes the possibility of a direct assessment of their total accumulation; however, information on PAH accumulation can still be gleaned indirectly from measurement of biomarkers of metabolic products such as the levels of fluorescent aromatic compounds in bile or PAH-DNA adduct levels. Krahn et al. (1987) analyzed bile from English sole from an urban waterway by gas chromatography/mass spectrometry (GC/MS) and showed that many of the fluorescent peaks detected by the high performance liquid chromatography (HPLC) method are metabolites of PAHs. In field studies, the levels of naphthalene-, phenanthrene-, and benzo[a]pyrene-like fluorescence have been measured in bile of a diverse group of fish species exposed to PAHs (Collier and Varanasi 1991; Hom et al. in press; Krahn et al. 1984). These studies and others (Lin et al. 1994; Statham et al. 1976) demonstrate that fish bile fluorescence is a reliable and easily measured marker of recent exposure to PAHs; however, the levels of bile fluorescence can decline relatively rapidly after the cessation of exposure, especially in feeding animals.

Results from a study of four species of marine fish exposed in the Exxon Valdez (Alaska) oil spill are indicative of this distribution pattern observed for fish (Hom et al. in press). Tissue levels of parent PAHs (determined by GC/MS) and metabolite fluorescence in bile (a semiquantitative HPLC method that measures PAH metabolite levels in bile) were measured in coho salmon (*Oncorhynchus kisutch*), pink salmon (*Oncorhynchus gorbuscha*), Pacific cod (*Gadus macrocephalus*), and Pacific halibut (*Hippoglossus stenolepis*) (Hom et al. in press). The concentrations of total parent PAHs in muscle of the halibut and cod were less than 1 ng/g of tissue (wet weight), although in salmon the concentrations were somewhat higher (most in the range of 0–20 ng/g). In contrast, fluorescent aromatic hydrocarbon (FAC) levels in bile of salmon and bottomfish ranged from 10 to 17,000 ng phenanthrene equivalents/mg bile protein, suggesting that substantial uptake of PAHs was occurring followed by rapid metabolic conversion to excretable metabolites. The levels of parent PAHs measured in muscle did not correlate with the concentrations of FACs in bile, which supports the facile metabolism of PAHs and shows that the metabolic capacity of these fish had not been exceeded. Moreover, these studies also showed that a broad range of waterborne PAHs from spilled oil were readily bioavailable to fish and that substantial uptake was occurring, with metabolism rapidly

Table 3. Occurrence of polycyclic aromatic hydrocarbons in marine organisms.

Species	Tissue	Area	Concentration (ng/g) BaP	Concentration (ng/g) Total PAH	d-w	Ref
Winter flounder	edible	New York Bight	7–21	14–315	*w*	1
Windowpane flounder	edible	NY Bight	1–4	18–536	*w*	1
Red hake	edible	NY Bight	nd–22	143–412	*w*	1
Crab1	edible	NY Bight	nd–1	52–1600	*w*	1
Lobster	edible	NY Bight	nd–25	129–367	*w*	1
Sea scallop	edible	NY Bight	nd–3	26–127	*w*	1
Mussel1	whole	S. Norway	3–91	500–12,845	d	2
Periwinkle	whole	S. Norway	2–8	595–1135	d	2
Limpet	whole	S. Norway	t–28	674–15462	d	2
Starfish	whole	S. Norway	nd	325–458	d	2
Sponge	whole	S. Norway	8	769	d	2
Algae1	upper	S. Norway	t–64	284–4665	d	2
Algae2	upper	S. Norway	t	445–2204	d	2
Algae3	upper	S. Norway	2–20	456–2964	d	2
Mussel1	whole	S. California	0.4–8.2	—	w	3
Mussel2	whole	S. California	<0.1–2.3	—	w	3
Oyster	whole	Chesapeake rivers	—	250–7610‡	d	4
Clam1	whole	Chesapeake rivers	—	740–15700‡	d	4
Mussel1	whole	Scotland	1–329	54–2803	w	5
Mussel1	whole	Vancouver, Canada	2–215	—	w	6
Mussel1	whole	East Coast U.S.	—	<25–3600	d	7
Mussel1	whole	West Coast Norway	—	>25000*	w	8
Periwinkle	whole	West Coast Norway	—	>20000*	w	8
Mussel1, clam2, clam3	whole	Gulf of Naples, Italy	5–21	198–205	w	9
Fish (14 species)	muscle	Gulf of Naples, Italy	nd–44	94–1930	w	9
Mussel3	whole?	Barcelona, Spain	—	190–5490	w	10
Crab2	whole?	Barcelona, Spain	—	80–930	w	10
Fish (tuna)	muscle	Barcelona, Spain	—	40–150	w	10

Fish (tuna)	liver	Barcelona, Spain	–	80-270	w	10
Mussel4	whole	Gulf of Mexico	–	121-7530	d	11
Tube worm	whole	Gulf of Mexico	–	73-1120	d	11
Mussel1	whole	Finnish Archipelago Sea	<0.5-5	<0.5-148	w	12
Fish2 (3 species)	muscle	Finnish Archipelago Sea	–	<0.5-33	w	12
Fish2 (3 species)	liver, gall bladder	Finnish Archipelago Sea	–	118-445	w	12
Lobster	digestive gland	Sydney Harbour, Nova Scotia	711-1430	13000-73000	w	13
Lobster	tail muscle	Sydney Harbour, Nova Scotia	27-43	295-2450	w	13
Oysters (not named)	whole	Gulf of Mexico, U. S. A.	–	<20-18,600	d	14
Mussel4	whole	Norway	–	5000-225000	d	15
Mussel4	whole	Northeast Italy	12-135	–	d	16
Mussel1	whole	Yaquina Bay, OR, U.S.A.	0.4-26 (avg)	–	*w*	17
Oyster	whole	South Carolina, U.S.A.	nd-1.6	nd-201	w	18
Oyster	whole	Elizabeth River, VA, U.S.A.	–	15000-60000‡	d	19
Lobster	tail	Nova Scotia	trace–40	20-2670	w	20
Lobster	hepatopancreas	Nova Scotia	0.4-1000	97-88100	w	20
Mussel4, oyster	whole	United States	–	<1-15,000#	d	21
Diatoms	whole	The Netherlands	25	454	d	22
Mussel1	whole	SE Australia	0.4-29	35-373 (AH)	d	23

Winter flounder (*Pseudopleuronectes americanus*), windowpane flounder (*Scophthalmus aquosus*), red hake (*Arophysate chuss*), rock crab (*Cancer irroratus*), lobster (*Homarus americanus*), sea scallop (*Placopeskin magellianicus*), mussel1 (*Mytilus edulis*), mussel2 (*Mytilus californianus*), periwinkle (*Littorina littorea*), limpet (*Patella vulgata*), starfish (*Asterias rubens*), sponge (*Halichondria panicea)*, algae1: bladder wrack (*Fucus vesiculosus*), algae2: knotted wrack (*Ascophyllum nodosum*), algae 3: toothed wrack (*Fucus serratus*), oyster (*Crassostrea virginica*), clam1 (*Rangia cuneata)*, clam2 (*Amigdala degussata*), clam3 (*Cardium edule*), mussel3 (*Mytilus galloprovincialis*), crab2 (*Macropipus tuberculatus*), tuna (*Thunnus thynnus*), tube worm (*Lamellibrachia* sp.), mussel4 (mussel unspecified; Mytilidae), lobster (*Homarus americanus*), mussel5 (*Modiolus modiolus*), diatoms (mainly *Navicula phyllepta*).

An approximate conversion of wet weight to dry weight concentration is ([PAH] dry weight = [PAH] wet weight × 5). ([PAH] dry weight/5) approximates wet weight concentration; nd, not detected; t, trace; d-w, dry weight or wet weight (italics indicate assumed wet weight); ‡, total resolved aromatics; #, site mean; *, naphthalenes, phenanthrenes, dibenzothiophenes; whole tissue, animal minus shell; AH, aromatic hydrocarbons.

References: 1, Humason and Gadbois (1982); 2, Knutzen and Sortland (1982); 3, Dunn and Young (1976); 4, Bender et al. (1986); 5, Mackie et al. (1980); 6, Dunn and Stich (1976b); 7, Farrington et al. (1983); 8, Grahl-Nielsen et al. (1978); 9, Cocchieri et al. (1990); 10, Porte and Albaiges (1993); 11, Wade et al. (1989); 12, Rainio et al. (1986); 13, Uthe and Musial (1986); 14, Wade et al. (1989); 15, Bjørseth et al. (1979); 16, Fossato et al. (1979); 17, Mix and Schaffer (1979); 18, Marcus and Stokes (1985); 19, Bender et al. (1988); 20, Sirota et al. (1983); 21, NOAA (1989) (see Mearns et al. 1991 for summary table); 22, Stronkhorst et al. (1994); 23, Murray et al. (1991).

removing the parent aromatic hydrocarbons (Hom et al. in press; Krahn et al. 1993).

On a longer time scale, Stein et al. (1993, 1995a) have shown that the levels of PAH-derived DNA adducts are dose responsive in fish exposed to benzo[a]pyrene or extracts of PAH-rich sediments and that the adduct levels increase on repeated exposure to PAHs. These findings indicate that levels of DNA adducts are measures of cumulative and relatively long-term exposure to polycyclic aromatic compounds. Hence, the measurement of bile fluorescence and DNA adducts can provide complementary information on short- and long-term exposure to PAH-like contaminants.

Very few studies of PAH accumulation in marine mammals exist; however, this general subject was reviewed by Engelhardt (1983). Uptake for these species probably occurs by absorption through the skin, ingestion, or inhalation, and accumulation is probably highest in lipid-rich tissue. Engelhard (1978) demonstrated that more naphthalene accumulated in the blubber and liver than in other tissues of ringed seals fed fish laced with ^{14}C-naphthalene in oil. This study also found that urine contained very high levels of water-soluble products, concluding that metabolism was active and that urine was a major route of elimination. Analysis of pinnipeds from Prince William Sound, Alaska following the Exxon Valdez oil spill showed low concentrations of primarily LPAHs in blubber but high concentrations of phenanthrene-like bile fluorescence in several animals (Frost et al. 1994; Hom et al. in manuscript). These findings support the hypothesis of efficient metabolism of PAHs by marine mammals and suggest that excretion of PAH metabolites occurs via bile as well as urine. In the Bering Sea, unsaturated hydrocarbons were found in the low ppm (2–20 μg/g wet wt) range in muscle, kidney, and liver of the spotted seal (*Phoca vitulina largha)*; however, this species contained up to 140 μg/g of saturated and unsaturated hydrocarbons in the blubber (Shaw et al. 1978). Engelhardt (1983) pointed out that some of these hydrocarbons could have a biogenic origin.

A. Sources of PAHs in Marine Organisms

Often the source of PAHs found in marine organisms is obvious, especially if an oil spill has recently occurred or if an identifiable point source is nearby. Many studies have found elevated PAHs in organisms near a known oil source. For example, sharply elevated concentrations of aromatic hydrocarbons (naphthalenes, phenanthrenes, and dibenzothiophenes) were found in field-collected mussels (*Mytilus edulis)*, periwinkles (*Littorina littorea*), sponges (*Halichondria panicea*), and sea anemones (*Tealia felina*) after an oil spill along the west coast of Norway (Grahl-Nielsen et al. 1978). These elevated concentrations (15–30 μg/g wet wt) declined to <1 μg/g in native organisms within several months after the spill. A study of lobsters (*Homarus americanus*) exposed to sediment high in PAHs from a

coking facility found the highest total PAH concentrations in the hepatopancreas (up to 88.1 μg/g wet wt) (Sirota et al. 1983). When compared to control lobsters, hepatopancreas concentrations were 74 to 908 times higher in lobsters from the most contaminated area. Even though PAH concentrations were relatively low (0.8–7.7 ng/mL) in discharge from a sewage treatment plant in Oslo, Norway, the authors concluded from the PAH profiles that mussels (*Mytilus edulis*) acquired their body burden of PAHs from this discharge (Kveseth et al. 1982).

Some studies have found that creosote-treated timber can be a source of PAHs to marine organisms. Tissue of mussels (*Mytilus edulis*) around creosote-treated piers contained elevated concentrations of benzo[a]pyrene, which declined with distance from the pilings (Dunn and Stich 1976b). The authors examined the gas chromatographic profiles of PAHs in mussels and nearby creosote-treated wood and found remarkably similar patterns in the chromatograms, indicating that the creosote-treated wood was probably the source in mussels. Dunn and Fee (1979) found that commercial lobsters (*Homarus americanus*) accumulated PAHs when kept in an impoundment area that was constructed with creosote-coated timbers. After 3 mon, the tail muscle of the animals in the pound facility contained from 3 times more phenanthrene to 482 times more benzo[k]fluoranthene than in control lobsters. Most of the HPAHs were 100–300 times more abundant in the impounded animals compared to control animals.

In certain areas in the Gulf of Mexico, where natural oil seepage is a common occurrence, many organisms coexist with elevated PAHs in water and sediment (Wade et al. 1989). Elevated concentrations of LPAHs (naphthalene, methyl- and dimethylnaphthalenes) were found in organisms, indicating the abundance of oil. In this study, the authors reported an interesting relationship between ^{13}C and ^{12}C in tissue. Tissues depleted in ^{13}C indicate carbon input that is chemosynthetic (vis-á-vis photosynthetic) in nature, which could be derived from methane that has been oxidized by bacteria. Mussels are known to have endosymbiotic methane-oxidizing bacteria that can supply a large percentage of a mussel's carbon input. Because methane, oil, and PAHs are expected to cooccur, an elevation of PAHs in tissues may also correlate with a depletion in ^{13}C because of the chemosynthetic activity of symbiotic bacteria in mussels. An association was found between elevated PAHs and reduced ^{13}C levels in mussels, indicating that this may be a useful monitoring tool of PAH exposure; however, further research is needed to establish its validity and utility.

Another study by Wade et al. (1988) determined that the source of PAHs to sediment and oysters from a wide area over the Gulf of Mexico was not sewage because of the lack of correlation with coprostanol, an indicator of sewage input. They also concluded that petroleum was not the source of PAHs to Gulf of Mexico sediments because the ratio of phenanthrene to anthracene ranged from 1 to 4.8; this ratio is closer to 50 in petroleum, which contains very little anthracene. They could not, however, rule out

weathered petroleum that had preferentially lost phenanthrene as the source of PAHs. Even though these two PAHs have very similar K_{ow}s, the solubility of phenanthrene is about 14 times higher than anthracene (1.0 vs 0.07 mg/L), making preferential loss a reasonable assumption.

A PAH profile with a high proportion of alkylated PAHs supports petroleum as a source, whereas a preponderance of the unsubstituted parent PAHs indicates combustion sources. An examination of PAHs in marine species from locations on the East Coast of the United States found low ratios of methylpyrene to pyrene (0.26), an indication that combustion PAHs were the main source for the organisms studied (bivalves, crabs, fish) (Pancirov and Brown 1977). The prevalence of unsubstituted compounds (in this case pyrene) indicates that combustion PAHs may be the major source. Broman et al. (1990) also reported that seston (inorganic and organic particulates >0.45 μm diameter, plus living plankton) samples from the Baltic Sea displayed a PAH profile consistent with that from combustion sources.

A few studies have shown that the sources of PAHs may be mixed. The bivalves (*Mytilus edulis*, *M. californianus*, and *Crassostrea* sp.) sampled in a "mussel watch" program for 1976–1978 contained elevated concentrations of aromatic hydrocarbons with phenanthrene distributions indicative of petroleum sources (Farrington et al. 1983). They also contained elevated pyrene, indicating a pyrogenic PAH source; hence, it was concluded that tissue burdens can show evidence of both types of PAHs.

Overall, these observations suggest that petroleum hydrocarbons are more readily available to organisms, whereas the pyrogenic PAHs are probably more strongly sorbed to particulates (e.g., sediment) and less available for uptake (Farrington et al. 1983). A hypothesis such as this can be assessed only by knowing the relative concentrations of PAHs in tissues and sediments, the relative metabolism of each PAH, and the partitioning behavior of these compounds. However, as stated earlier, there is some evidence that sedimentary PAHs do not partition according to expected models.

B. Seasonal Effects on Tissue Concentrations

Several researchers have noticed that PAH concentrations in marine organisms appear to show seasonal variation, which may be caused by a number of factors. Both oysters (*Crassostrea virginica*) and clams (*Rangia cuneata*) sampled in the fall contained about 2–3 times more aromatic hydrocarbons than those sampled at the same sites during the spring season (Bender et al. 1986), leading to the hypothesis that differences resulted from the spawning cycle. Oysters spawn during the summer and clams during late fall and early winter, and both were at their high point of lipid and glycogen reserves during sampling. Higher benzo[a]pyrene concentrations were found by Dunn and Stich (1976b) in mussels (*Mytilus edulis*) from waters around

Vancouver, Canada during the winter (versus summer), which was attributed to variability in sewage and storm drain discharge. A winter maximum of benzo[a]pyrene was also found by Fossato et al. (1979) in mussels (*Mytilus* sp.) collected in northeast Italy. Conversely, Mix and Schaffer (1979) found no seasonal differences over their 1976–1977 sampling years in Yaquina Bay, Oregon; however, they did find a peak abundance of benzo[a]-pyrene in *Mytilus edulis* during their winter their 1977–1978 sampling. Farrington et al. (1983) also described very large seasonal differences in PAH concentrations in mussels (*Mytilus edulis*) from the East Coast of the US. They found elevated concentrations of PAHs (300–1300 ng/g dry wt) during winter and spring, declining concentrations during summer, and low concentrations in fall (< 100 ng/g) in mussels from Narragansett Bay, Rhode Island. They attributed these differences to variability in filtration rate, spawning activity, variable phytoplankton ingestion, microbial activity, environmental concentrations, chemical forms (particulate, colloidal, free), chemical species, and other related factors. It appears that spawning activity may be an important determinant of variable PAH tissue burdens; however, other factors that may vary with season, such as filtration rate and PAH input, need to be explored further.

C. Distribution Within Organisms

As do most organic contaminants, PAHs accumulate in certain tissues with the highest proportions found in the liver of vertebrates or the hepatopancreas of invertebrates. In general, lipid-rich tissues preferentially accumulate parent PAHs because of their strongly hydrophobic nature. Metabolites of biotransformed PAHs tend to accumulate in certain vertebrate tissues, such as bile and liver. In invertebrates, metabolite concentrations are generally highest in the hepatopancreas.

Many studies have explored the distribution of PAHs in tissues, and most examined their occurrence in reproductive tissue. For example, polychaetes (*Neanthes arenaceodentata*) of both sexes rapidly took up naphthalenes (naphthalene, methylnaphthalenes, and dimethylnaphthalenes) and achieved equal tissue burdens, but males released these compounds gradually over time, whereas females retained the naphthalenes for up to 17 d and then released them abruptly when their eggs were spawned (Rossi and Anderson 1977) . This implies that these naphthalenes accumulated in the lipid-rich eggs and were not available for elimination until spawning. Further research showed that naphthalenes were passed to the zygotes, retained until the trochophore stage, and then lost as the trochophores developed into 18-segment juveniles (Rossi and Anderson 1977). This reduction in naphthalenes was not caused by growth dilution, because weights were similar between trochophores and juveniles but may have been caused by utilization of yolk reserves. Ellis et al. (1993) also found that PAHs were associated with gametes and that eggs and sperm of oysters (*Crassostrea*

virginica) exhibited PAH concentrations up to 5 times higher than that for somatic tissue. This study also found that the amount of gonadal tissue was the most important determinant of PAH body burden and that oysters may expel up to 50% of their total PAH body burden in a single spawning event. Conversely, Mix et al. (1982) found that benzo[a]pyrene did not preferentially accumulate in the gonad of mussels (*Mytilus edulis*) that were examined from January through May and presumably included the spawning period. This finding is supported by Lee et al. (1976), who found that after 2 d of exposure to naphthalene, methylnaphthalene, and benzo[a]pyrene in food, the crab *Callinectes sapidus* accumulated most of these compounds in the gill, blood, and hepatopancreas, with a small amount in the gonad. These studies cannot rule out possible redistribution of PAHs among tissues, which may be an important factor in longer-term studies. When sexually mature English sole (*Parophrys vetulus*) were exposed to either benzo[a]pyrene or naphthalene, the parent hydrocarbon predominated in the lipid-rich gametes (Hose et al. 1981; Reichert and Varanasi 1982; Varanasi et al. 1982).

Even though both the alkane and aromatic fractions were higher in somatic tissue than in gonadal tissue (on a weight specific basis) in *Mytilus edulis* and *M. californianus*, it was more likely for the aromatic fraction to dominate over the alkane fraction in the gonadal tissue (DiSalvo et al. 1975). Aromatics were enriched in the eggs, especially during the nonspawning season. One study that did not examine reproductive tissue found that excised gill of mussels (*Mytilus edulis*) was able to take up more naphthalene from seawater than excised mantle or adductor mussel, which was attributed to a micellar layer (Lee et al. 1972). Alternatively, the gill may have absorbed more hydrocarbon because of its larger surface area per gram of tissue and continued renewal of hydrocarbon-laden seawater at its surface from ciliary action.

The distribution of PAHs in tissues may be of considerable interest to consumers of seafood, especially when muscle tissue is compared to internal organs. Dunn and Fee (1979) examined the digestive gland (a.k.a. "tomalley," a delicacy in some cultures) of commercial lobsters (probably *Homarus americanus*) and found 7–11 times more PAHs than in tail muscle. The highest value of benzo[a]pyrene found in lobster hepatopancreas was 2300 ng/g. This finding was confirmed by Uthe et al. (1984), who reported 58–250 times more pyrene and benzo[a]pyrene, respectively, in lobster hepatopancreas than in tail muscle. Additional studies of edible shellfish have found similar patterns of distribution. Shrimp (*Penaeus aztecus*) accumulated the highest concentrations of naphthalenes in the digestive gland (Neff et al. 1976), and crab (*Callinectes sapidus*) accumulated the highest levels of parent benzo[a]pyrene and metabolites in the hepatopancreas, the presumed site of metabolism (Lee et al. 1976).

Disposition of PAHs and their metabolites in tissues is often influenced by lipid content and metabolic ability; thus, it is not surprising that the hepatobiliary system usually accumulates high levels of PAHs and their

metabolites because liver is the tissue that usually has the highest levels of lipid and xenobiotic-metabolizing enzyme activities (Buhler and Williams 1989). Species with a high hepatic lipid content, such as the grunt (*Haemulon sciurus*) and the spiny dogfish (*Squalus acanthias*), can accumulate high levels of PAHs such as phenanthrene in the liver as compared to muscle (Solbakken and Palmork 1980; Solbakken et al. 1982); however, elevated lipid in muscle will lead to a different distribution. For example, in a fish with a high lipid content in muscle, such as rainbow trout (*Oncorhynchus mykiss*), the ratio of the percentage dose of phenanthrene in liver to that in the muscle was 0.28, whereas in the coalfish (*Pollachius virens*), which has high lipid reserves in the liver, this ratio was 12 (Solbakken and Palmork 1980; Solbakken et al. 1979). Interestingly, in rainbow trout and coalfish, more than 3% of the phenanthrene dose was found in the gallbladder at 24 hr postinjection, whereas in the spiny dogfish only 0.07% of the dose occurred in this organ. This species difference in tissue distribution can be explained by the apparently lower P450 activity in elasmobranchs as compared to teleosts.

Because tissues have different capacities for biotransformation, we also find variable proportions of metabolites throughout the body. In English sole (*Parophrys vetulus*) exposed via diet or sediment, the proportion of naphthalene metabolites in various tissues ranged from 1% to 87% of total naphthalene-derived compounds present 24 hr after dosing (Reichert and Varanasi 1982; Varanasi and Gmur 1981). When fish were exposed to benzo[a]pyrene, the relative distribution of benzo[a]pyrene-derived radioactivity (primarily metabolites) in fish tissues was bile ≥ liver > skin > muscle regardless of species, route, or frequency of exposure (continuous versus single) (Varanasi and Stein 1991). In general, the ratio of metabolites to parent PAHs in fish tissues generally increases with time for a single exposure and is PAH independent (Reichert and Varanasi 1982; Varanasi and Gmur 1981; Varanasi et al. 1979, 1989a); these findings were based on results obtained with naphthalene, phenanthrene, and benzo[a]pyrene, which are markedly different in size and hydrophobicity.

Temperature can also influence the distribution of metabolites. In a study with gulf toadfish (*Opsanus beta*), Kennedy et al. (1989) found a higher bile-to-liver ratio of benzo[a]pyrene metabolites when fish were exposed at a higher temperature. They concluded that this resulted from an increase in transfer of metabolites from liver to bile as a result of increased metabolism.

VII. Accumulation in Relation to Environmental Concentrations

A. Patterns of Accumulation

Many studies have shown that marine organisms can accumulate PAHs from the environment. It is not so much a question of "if an animal will accumulate PAHs" but rather "how much it will accumulate" given the environmental concentrations, controlling factors of bioavailability, time

scale, and organism physiology. Bioaccumulation patterns of PAHs in marine organisms may differ based on many factors including, but not limited to, exposure medium, uptake rate, metabolic capability, lipid content, and feeding strategy (Adams 1987; Meador et al. 1995; Roesijadi et al. 1978a; Schrap and Opperhuizen 1990; Varanasi et al. 1985). Each of these factors has to be considered whenever accumulations of PAHs are considered and compared. Because biotransformation is one of the more important processes, evaluation of metabolic capacity and disposition of parent compounds must be included in any study of PAH bioaccumulation to accurately assess total uptake.

Because PAHs are hydrophobic, the lipid content of an organism can have a large effect on tissue concentration (Mackay 1982; Stegeman and Teal 1973), which is generally predictable. By knowing the environmental concentration, organic carbon content, and tissue lipid content, the equilibrium tissue concentration for a PAH may be predicted with the K_{ow} (Lee 1992; Lee et al. 1993; McCarty 1986). As determined by fugacity calculations (thermodynamics), an aquatic organism should come into equilibrium with its exposure concentration, given sufficient time (Mackay and Paterson 1981). Of course, this assumes that the exposure concentration is constant, the environmental matrices are in equilibrium with each other, and diffusive loss is the only form of elimination. If the rate of uptake is constant and the compound is continually metabolized or excreted, the organism may come to an apparent equilibrium, which would be below the predicted dynamic equilibrium. This is referred to as steady state because of the unchanging tissue concentrations. As mentioned in Section V on uptake and elimination, myriad factors affecting the rates of uptake and elimination that can have a direct bearing on steady-state tissue concentrations.

A few studies have shown patterns contrary to those that were expected. For example, Dunn's (1980) field study of mussels (*Mytilus edulis*) and algae (the seaweed *Fucus* sp.) found progressively lower concentrations of PAHs in mussels as hydrophobicity increased. It was assumed that this pattern resulted from the organism's uptake of these compounds from water and the declining solubility of PAHs as hydrophobicity increased; however, sediment concentrations, bioavailability, and bioaccumulation factors need to be considered. This pattern of decreasing PAH in tissue with increasing hydrophobicity was not observed for the seaweed, which was possibly caused by trapping particles laden with PAH. Lee et al. (1978) found that the BCFs for the more hydrophobic PAHs in oysters (*Crassostrea virginica*) after 2 d exposure to an oil slick were lower than those for the LPAHs, which is opposite what was expected. This is possibly due to lowered water concentrations for the more hydrophobic PAHs and a short time period for attainment of steady-state concentrations. Even after 4 mon of exposure to oil-spiked sediment, winter flounder (*Pseudopleuronectes americanus*) displayed bioaccumulation factors (muscle to sediment concen-

trations) that declined with increasing hydrophobicity (Hellou et al. 1994). This type of pattern may reflect the combined effects of rapid metabolism of all PAHs and reduced bioavailability of the more hydrophobic compounds.

Accumulation in animals can be highly variable, even in apparently similar individuals of a species from the same site. For example, Wade et al. (1988) reported coefficients of variation (CV) for triplicate analyses of PAHs in the tissues of oysters from sites in the field to be generally much higher (CV = 10%–175%) than that seen for a homogeneous standard reference material (CV = 20%) (e.g., oyster tissue from the U.S. National Institute of Standards and Technology), indicating variability in accumulation among individuals from a given site. Lipid and water content normalization can help eliminate some of the variation in PAH concentrations among individuals; however many factors such as age, reproductive condition, and ventilatory rate can affect intraspecific variability.

Many studies have shown a basic pattern of high PAH accumulation from areas with high environmental concentrations. Oysters (*Crassostrea virginica*) suspended above sediment contained high levels of PAHs when compared to oysters exposed to sediments with much lower concentrations (Pittinger et al. 1985). Similar results were obtained by Gardner et al. (1991) when they exposed oysters (*Crassostrea virginica*) to contaminated Black Rock Harbor sediment that produced PAH levels 10–75 times higher than those observed in oysters exposed to reference sediment. Additionally, Farrington et al. (1983) and Murray et al. (1991) reported that fossil fuel hydrocarbons (as well as other contaminants) were elevated about an order of magnitude in mussels (*Mytilus edulis*) from contaminated areas compared to those from reference areas.

Correlations between PAHs in the environmental matrix and tissue can be useful in assessment of environmental exposure. One study found a strong gradient of PAH concentration both in sediment and in mussel (*Mytilus edulis* and *Modiolus modiolus*) tissue up to several kilometers away from a ferro-alloy smelter (Bjørseth et al. 1979). Near the smelter, the sediments produced a tissue to sediment ratio of about 2 (dry/dry wt) for total PAH. Interestingly, benzo[b]fluoranthene was the dominant PAH in mussels at all stations; however, in sediment it was only the fifth most abundant PAH (although the four most abundant PAHs were less water soluble). In another study, Dunn (1980) found a high correlation ($r = 0.78$) between mussels (*Mytilus edulis*) and sediment benzo[a]pyrene concentrations.

A few studies have examined tissue–sediment correlations of PAHs in fish, usually with indicators of exposure other than concentrations of parent compounds in tissue. Studies with English sole (*Parophrys vetulus*) have shown a dose–response relationship between exposure to benzo[a]pyrene or PAH-rich sediment extract and benzo[a]pyrene-like bile fluorescence levels (Collier and Varanasi 1991). Moreover, a wide range of fish species from sites with increased sediment concentrations of PAHs have demonstrated

exposure to PAHs by exhibiting high levels of PAH-like fluorescence in bile (Collier et al. 1993; Stein et al. 1992; Wirgin et al. 1994). The levels of PAH-like DNA adducts and PAH-like bile fluorescence in bile of the territorially restricted oyster toadfish (*Opsanus tau*) captured in the creosote-contaminated Elizabeth River, Virginia showed highly significant linear correlations with the levels of parent PAHs in the sediment over a broad range of PAH contamination (10^5), indicating a dose-dependent relationship (Collier et al. 1993) (Figs. 13 and 14). Similar correlations were found between sediment PAH levels and hepatic DNA adduct levels in eels from waters around Amsterdam (van der Oost et al. 1994), Atlantic tomcod (*Microgadus tomcod*) from river systems of the northeast coast of North America (Wirgin et al. 1994), and in three species of flatfish from Puget Sound, Washington (Stein et al. 1992). It is noteworthy that fish displayed increasing cataracts, reduced biomass, and reduced numbers of individuals, without noticeable tissue concentrations of parent hydrocarbons, over an increasing gradient of PAH-contaminated sediments in the estuary of the Elizabeth River, Virginia (Huggett et al. 1987). Several other field studies have also shown statistical associations between exposure to PAHs and biological effects (e.g., lesions, reproductive impairment) in marine fish species (Johnson et al. 1988, 1993; Landahl et al. 1990; Myers et al. 1994).

In another recent study utilizing fish, Goksøyr et al. (1994) found a

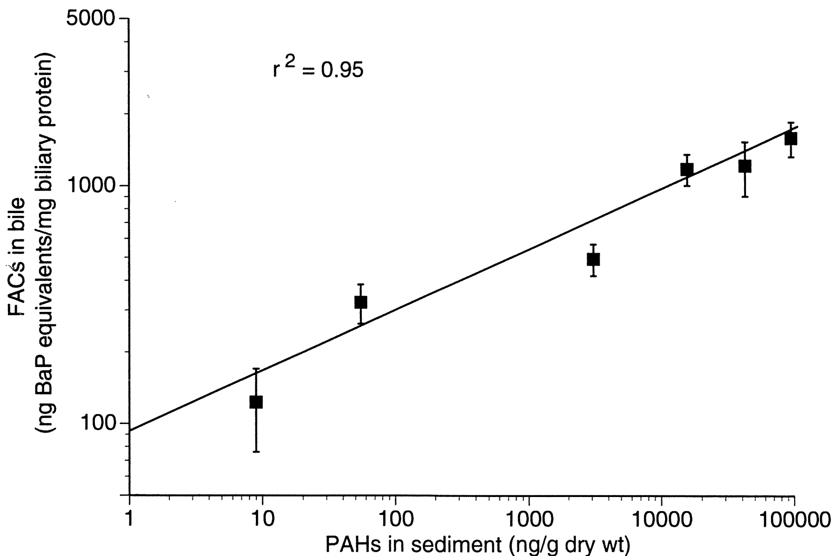

Fig. 13. Relationship between the level of fluorescent aromatic compounds (FACs) in the bile of oyster toadfish (*Opsanus tau*) and concentration of total PAHs in sediments. Values are mean and standard error of the mean. Samples are from the Elizabeth River, Virginia, U.S.A. (Redrawn from Collier et al. 1993.)

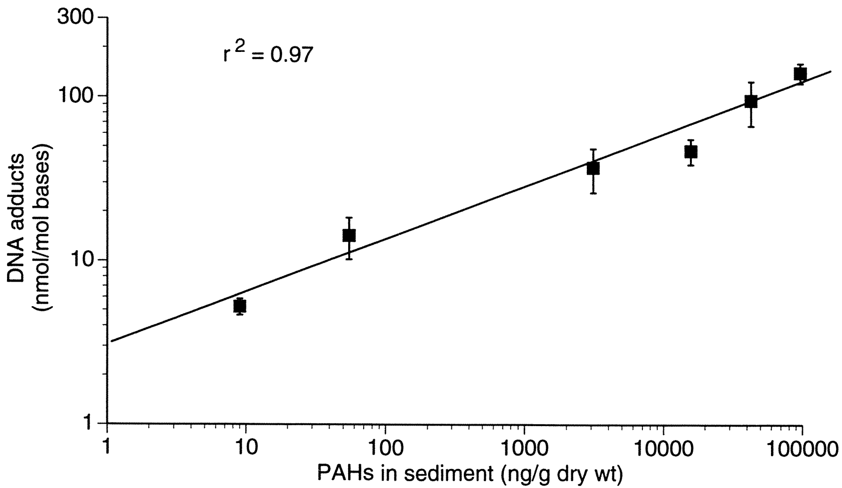

Fig. 14. Relationship between the level of DNA adducts in the liver of oyster toadfish (*Opsanus tau*) and concentration of total PAHs in sediments. Values are mean; error bars are standard error of the mean. Samples are from the Elizabeth River, Virginia, U.S.A. (Redrawn from Collier et al. 1993.)

gradient of parent aromatic hydrocarbons in caged juvenile Atlantic cod (*Gadus morhua*) placed in a polluted fjord (Sørfjorden, Norway). Additionally, Hellou et al. (1994) found an increase in parent PAHs in winter flounder (*Pseudopleuronectes americanus*) muscle when exposed to a gradient of oil-spiked sediments in the laboratory. These studies, and earlier research, show that direct measurement of PAHs in tissues of fish is possible; however, the information obtained will substantially underestimate the total accumulation.

Biomagnification, an increase in tissue concentration over two or more trophic levels, is generally not expected to occur for PAHs, except possibly in species from the lower trophic levels that are not able to effectively metabolize these compounds. This statement is probably true because most food chains usually involve a vertebrate, which in most cases can actively biotransform PAHs. This ability to degrade PAHs leads to a short half-life for these compounds in tissue that prevents accumulation. Other food webs that include only invertebrates (e.g., predatory molluscs and polychaetes that prey on other polychaetes and molluscs) would be a good test of biomagnification of PAHs. Biomagnification of contaminants, including PAHs, has been reviewed by Suedel et al. (1994).

One study attempting to demonstrate the importance of dietary accumulation found that the grass shrimp (*Palaemonetes pugio*) contained 15 to 24 times more dimethylnaphthalene than the brine shrimp (*Artemia* sp.) it was ingesting (Dillon 1981). These results indicate that food chain transfer may

occur and that there is a potential for biomagnification in some species. In these experiments, individuals of *Artemia* sp. were exposed to the PAH for only 8 hr and the grass shrimp were exposed to the PAH-laden prey for 32 d. It should be kept in mind that to assess food chain transfer both predator and prey tissue burdens should be at steady state with respect to their source to minimize disparity in the calculated tissue ratio.

Even though parent PAHs may not be biomagnified, very little research has examined accumulation of metabolites from prey. After being fed polychaetes (*Nereis virens*) containing radiolabeled benzo[a]pyrene (14%) and accompanying metabolites (86%) or pure benzo[a]pyrene for 24 hr, both the parent compound and its metabolites were detected in winter flounder (*Pseudopleuronectes americanus*), suggesting that metabolites were taken up (McElroy and Sisson 1989). Subsequently, McElroy et al. (1991) demonstrated that absorption of benzo[a]pyrene-7,8-diol, the precursor to the ultimate carcinogenic form of benzo[a]pyrene, was similar to adsorption of the parent compound when fed to winter flounder. This suggests that the bioavailability of certain carcinogenic PAH metabolites may be of concern if elevated levels of metabolites are present in prey species. Another laboratory study found that winter flounder (*Pseudopleuronectes americanus*) fed contaminated mussels in the laboratory contained the same compounds as those occurring in mussels (Gardner et al. 1991). Additional research assessing the bioaccumulation of PAH metabolites from ingested prey will add greatly to our understanding of this very important process of trophic transfer.

Ideally, the best species to use for monitoring bioavailable PAH contamination in the environment would be a polychaete or bivalve species with minimal metabolic capacity for PAHs. It should be noted, however, that even though species with low biotransformation activity may be the best candidates for monitoring PAH bioaccumulation, many factors affecting uptake and elimination, including factors that impair animal health, will influence PAH body burden. This is an important consideration for any study of PAH bioaccumulation in field-collected organisms.

The abundance of articles in this review that examined PAH concentrations in bivalves indicates their utility as a taxon with a high likelihood to accumulate these chemicals and limited ability to eliminate their acquired burden. Because of their reduced metabolism of these compounds, bioaccumulation in bivalves allows us to speculate that other organisms in the vicinity may also be accumulating these compounds. Using bivalves as indicators can be accomplished either by sampling native populations, which may be eliminated within their normal range if tissue concentrations are too high, or by placing animals from similar uncontaminated environments into areas of interest. For example, mussels (*Mytilus californianus*) from unpolluted waters that were placed in San Francisco Bay rapidly accumulated hydrocarbons ($\approx 3.3\ \mu g$ total hydrocarbons g^{-1} dry mussel d^{-1}) (Disalvo et al. 1975). Sampling of *in situ* mussels and oysters as indicators of organic and metal/element contamination has been an ongoing project for

many years and is commonly referred to as "mussel watch" programs (Farrington et al. 1983; O'Connor 1991; O'Conner et al. 1994; Wade et al. 1988).

B. Bioconcentration Factors

The bioconcentration factor (BCF) is the ratio of the tissue concentration of a particular chemical to its water concentration. It should be kept in mind that the BCF is relevant only for accumulation from water; to compare among BCFs it is important to establish that water is the only route of uptake. At equilibrium, the BCF generally increases with increasing chemical hydrophobicity because of the increased fugacity or tendency of the chemical to partition into the animal's lipid rather than stay in solution. Many authors have claimed that the BCF can be predicted with such physico-chemical factors as octanol–water partitioning and solubility (Chiou et al. 1977; Mackay 1982; Pruell et al. 1986; Veith et al. 1979); however, metabolized compounds may skew the predictions.

The expected BCF for a variety of neutral organic compounds, such as a PAH, can be determined with the following formula: BCF = 0.046 K_{ow} (McCarty 1986). Octanol is generally a good predictor of lipid partitioning and the coefficient 0.046 indicates that the average fish is approximately 4.6% lipid, which is a reasonable approximation. While this formula was developed using fish data (based on wet weights), its utility for other taxa is uncertain. Steady-state BCFs, mainly LPAHs, for an infaunal, deposit-feeding amphipod (*Diporeia* spp.) known to have high lipid content (8.0% wet wt) and a weak or nonexistent ability to metabolize PAHs, were generally higher than those calculated with the above mentioned formula for fish (even after correcting for lipid content) (Landrum 1988; Landrum et al. 1994). Although not done routinely, expressing the BCF in terms of organism lipid content should be useful for reducing the variability observed between individuals within a species.

Accumulation can be a function of PAH hydrophobicity, but time of exposure and metabolic capacity must also be considered. The day 7 BCFs for clams (*Macoma inquinata*) exposed to sediment containing phenanthrene, chrysene, dimethylbenz[a]anthracene, and benzo[a]pyrene, varied 131-fold and were strongly correlated to molecular weight (and hydrophobicity) (Roesijadi et al. 1978a). The same pattern was found for naphthalene BCFs (naphthalene to trimethylnaphthalene) in a clam (*Rangia cuneata*), which showed a dramatic increase (2.3 to 26.7) as molecular weight (and hydrophobicity) increased (Neff et al. 1976).

The BCF at steady state is a function of uptake (k_1) and elimination (k_2) and can be predicted with k_1/k_2. Highly hydrophobic PAHs can produce high concentrations in tissues and high BCFs, even when environmental concentrations are very low. For species that do not metabolize PAHs, the BCF should increase with K_{ow}; however, if the ratio of k_1 to k_2 does not increase, the generalized pattern will not hold. McKim et al. (1985) found that the positive correlation between BCF and K_{ow} was controlled by the

declining elimination rate because the rate of uptake was relatively constant over compounds with log K_{ow}s ranging from 2.8 to 6.2. Future hypothesis testing will help elucidate the theory that the correlation between BCF and K_{ow} for an organic contaminant is a function of the rate of elimination. It would be very useful to know how the rate of elimination varies over a range of hydrophobic PAHs and to what degree these rates may impact the correlation between the BCF and K_{ow}.

Even when the balance between uptake and elimination is expected to be correlated with K_{ow}, the observed BCFs may not always be as predicted. When the total PAH in porewater was used to calculate the BCF, a deviation from expected was observed in an infaunal amphipod (*Rhepoxynius abronius*), which presumably received most of its body burden of all PAHs via porewater. Instead of a correlation of K_{ow} and BCF, a leveling-off of the BCF for the most hydrophobic compounds was observed (Fig. 2) (Meador et al. 1995). This plateauing of the BCF indicates that either the tissue concentration was less than expected or the water concentration was greater than expected, or, more specifically, the organisms were accumulating only a portion of the total PAH in solution. The less than expected tissue concentrations for the HPAHs could have been due to increased metabolism for these PAHs as hydrophobicity increased; however, such large increases were not expected. Because the ratio of uptake to elimination (k_1/k_2 = BCF) was apparently the same over the LPAHs for the two species in this study (*R. abronius* and *Armandia brevis*), and they differed only twofold in their ability to metabolize benzo[a]pyrene, the rate of elimination was not expected to change for only one of the species over the HPAHs. One possibility is that the amphipod was responding to only the free concentration of PAH, which was decreasing on a percentage basis as hydrophobicity increased. This association of PAH and DOC caused the apparent concentration of PAH in porewater to be higher than what was bioavailable to the amphipod, which resulted in a smaller than expected BCF. When the BCF was computed with free PAH, it became more positively correlated to K_{ow} and much closer to expected.

Several studies have shown rapid attainment of steady-state BCFs in marine organisms exposed to PAHs in water. For example, similar values (BCFs ≈ 50) were calculated for *Mytilus edulis* after 4 hr of exposure to labeled naphthalene and after 4 wk exposure to unlabeled naphthalene, indicating rapid equilibration with exposure concentrations (Widdows et al. 1983). Rapid accumulation was also noted by McLeese and Burridge (1987) in clams (*Mya arenaria)*, mussels (*Mytilus edulis)*, shrimp (*Crangon septemspinosa*), and polychaetes (*Nereis virens*) exposed for 4 d to several PAHs in water. They concluded that these species had accumulated sufficient PAH to have BCFs within a factor of 2–5 of steady-state BCFs (based on k_1/k_2). Clams (*Tapes japonica*) placed in the Port of Osaka, Japan to measure accumulation at natural levels of PAHs in seawater exhibited BCFs of 12,800 for pyrene, 2,800 for benzo[b]fluoranthene, 5,700 for benzo[k]fluoranthene, and 4,600 for benzo[a]pyrene after 7 d exposure

(Obana et al. 1983). These are similar to but generally higher than (especially pyrene) than those found in their laboratory experiments, which probably results from ingestion of particles (e.g., sediment, phytoplankton, and zooplankton) with sorbed PAHs.

Determination of BCFs in field-collected animals is uncommon, possibly because it is difficult to assure water-only exposure and accurately determine temporally variable concentrations. One study reported variable BCFs in the range of 7×10^3 to 3×10^4 in an urban area and 1×10^5 to 5×10^5 in an oil refinery area for mussels (*Mytilus edulis planulatus*) from southeast Australia (Murray et al. 1991). Interestingly, the BCFs for benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[a]pyrene were 10–44 times higher in individuals collected from the oil refinery sites versus those in animals collected from the urban sites. These large differences in BCF may result from variability in environmental concentrations or possibly be a consequence of different PAH sources producing differential bioavailability. It is noteworthy that at both locations the ratio between particulate-associated PAH and dissolved PAH in a liter of water was generally between 2 and 10, depending on the PAH, indicating enrichment of the compound on particles. Additionally, concentrations of the three PAHs in particulates from the urban sites were generally 3–4 times higher than those particulates measured from the oil refinery sites. The BAFs were calculated by summing the PAH concentrations in the dissolved and particulate phases, and thus much of the BCF denominator resulted from the particulate phase. As discussed, sediment-associated PAHs from urban sites are generally from combustion sources and have been reported to exhibit reduced partitioning. The large contribution of the particulate phase to the environmental concentration used to calculated the BCF, and the reduced partitioning found at many urban sites, suggest that less PAH may be accumulated from particulates at these sites, which would cause the denominator in the BCF equation to be overestimated and hence the BCF underestimated.

Bioconcentration factors for fish are very difficult to calculate because extensive biotransformation results in the rapid disappearance of parent PAHs from tissues. Measurement of tissue burdens of parent compounds normalized to water concentrations will severely underestimate the BCF. As described earlier, alternate measurements of PAH accumulation may be more useful in assessing exposure.

C. Bioaccumulation Factors

The bioaccumulation factor (BAF) is generally computed as the ratio between the contaminant concentrations in tissue and multiple external sources (e.g., sediment, water, and diet) and is useful in determining the tendency of hydrophobic compounds to accumulate in tissue. A seldom-used term, *dietary accumulation*, is used to determine the ratio between the concentration of a contaminant in an organism and its food. The BAF can be just tissue/sediment concentration, which is also known as the tissue-to-

sediment ratio (TSR); however, this ignores water and prey as sources. The BAF has been recently modified to include lipid-normalized tissue and organic carbon-normalized sediment concentrations. This ratio has been called by many different names, including the accumulation factor (AF) (by most authors), biota–sediment accumulation factor (BSF) (Di Toro et al. 1991; Thomann et al. 1992), equilibrium partitioning factor (PF), and the BAF_{loc} (Meador et al. 1995). This has also been calculated as the inverse ratio and is called the APF (apparent preference factor) (McElroy and Means 1988). We favor the term BAF_{loc} because it is consistent with related nomenclature and historical usage of the term. Because of the lack of convention, however, when discussing a study we will use the term stated in that paper. The reader should also notice whether these terms are expressed in terms of dry or wet weight. This is important because sediment dry to wet weight ratios often fall between 0.5 and 0.8, whereas dry to wet weight ratios for organisms are generally between 0.1 and 0.25. Therefore, a BAF expressed in terms of dry or wet weight can be quite different, whereas it does not matter for BAF_{loc}, as long as there is consistency within the numerator and denominator (e.g., dry weight for both sediment concentration and f_{oc}). Researchers are urged to adopt the dry weight convention, or at least supply the information for the reader to interconvert, because of the inherent variability in wet weights.

Determination of lipid- and organic carbon-normalized BAFs may be useful in assessing whether a test species has reached equilibrium tissue concentration. This is especially true for short-term uptake studies using different compounds that produce differential partitioning. By normalizing PAH tissue concentration to lipid concentration (ng tissue PAH/g lipid) and the sediment concentration to organic carbon concentration (ng sediment PAH/g OC), for any given PAH, the ratio at equilibrium should be the same, which has been purported to be about 1.7 (Lee et al. 1993). Because it is assumed that PAHs display similar partitioning behavior into lipid and organic carbon, normalizing tissue and matrix concentrations to these factors should produce a similar ratio over a range of hydrophobic PAHs. Because of this feature, we may be able to predict the equilibrium BAF across a series of hydrophobic PAHs, regardless of the route of uptake. By multiplying the ratio between organism lipid and sediment TOC with the theoretical maximum BAF_{loc} of 1.7, one can predict bioaccumulation based on sediment concentration. If average values are used (5% for tissue lipid and 1% for sediment TOC), it appears that, in general, PAH tissue concentrations for a nonmetabolizer should be about 10 times higher than sediment, regardless of PAH hydrophobicity. Of course, as the ratio of lipid to TOC varies, the BAF will change accordingly. Contrary to predictions for BCFs, this relationship implies that there is a lack of correlation between BAFs and hydrophobicity, which may be explained by fugacity. This relationship for BAFs also implies that the uptake and elimination kinetics are the same for each species, which may be explored with hypothesis testing. It should be noted that many factors, such as reduced bioavail-

ability and metabolism, can produce a BAF_{loc} (or BAF) that is less than expected.

Caution is also warranted with regard to the methods to determine lipid and organic carbon concentrations in tissue and sediment. A good discussion concerning the method for both of these variables can be found in Lee et al. (1993). For lipids, a method that extracts both polar and neutral lipids with a combination of solvents such as chloroform and methanol is preferred (Bligh and Dyer 1959; Herbes and Allen 1983). Total and dissolved organic carbon should be determined with an instrument that specifically measures carbon, utilizing methods such as those found in Standard Methods (APHA 1989) or those from the American Society for Testing and Materials (ASTM 1994).

Several studies have reported BAFs for sediment-dwelling organisms. Foster and Wright (1988) reported BAF (actually BAF_{loc}) values in the range of 0.5–2.2 for a clam (*Macoma balthica*) and 0.4–1.8 for a polychaete worm (*Nereis succinea*) collected from several sites (sediment >39% silt and >0.5% TOC) in Chesapeake Bay. Both species are sediment ingesters, which may explain the similar accumulation factors. Bioaccumulation was also studied in a deposit-feeding clam (*Macoma nasuta*) exposed for 28 d to field-contaminated sediments from near the Los Angeles County, California sewer outfalls (Ferraro et al. 1990). AF values for pyrene, benz[a]anthracene, chrysene, benzo[b,[k]]fluoranthene, and benzo[a]pyrene were generally in the range of 0.2–1.0. Another study of PAH bioaccumulation from field-contaminated sediment found day 10 HPAH BAF_{loc}s for the polychaete *Armandia brevis* in the range of 0.21–0.80 (median, 0.47), which when corrected for metabolism were close to the theoretical maximum of 1.7 (Meador et al. 1995). The steady-state partitioning factors for a deposit-feeding clam (*Macoma balthica*) were determined by Foster et al. (1987) to be 0.78 for naphthalene and 0.17 for chrysene, when based on uptake and elimination rate constants. Other studies with sediment-dwelling polychaetes and clams also have found BAF_{loc}s ranging from 0.5 to 3.8 (Augenfeld et al. 1982; Brannon et al. 1993; Weston 1990), and AFs for PAHs similar to those discussed have been reported for freshwater invertebrates (Bierman 1990).

In contrast, Lake et al. (1985) found very low BAFs ([tissue]/[sediment]) for PAHs in the polychaete *Nereis virens* (0.02–0.07) exposed to field-collected sediment from Black Rock Harbor, Connecticut, which may be even lower when expressed as BAF_{loc}s because lipid content in tissue is generally higher than TOC in sediment. The authors pointed out that feeding by the worms was poor and thus water may have been the major route of PAH uptake. Because they also reported partitioning (K_p) values ([sediment]/[water]) that were 5–50 times higher than expected, we can conclude that water concentrations of PAHs were much lower than expected, which may be responsible in part for the lower than expected BAFs.

Some studies report bioaccumulation factors higher than expected when sediment organic carbon falls below approximately 0.5% (Foster and

Wright 1988; Weston 1990). In the lugworm (*Abarenicola pacifica*), Weston (1990) found higher BAF_{loc}s (our calculations, assuming 5% lipid) for a low-TOC sediment (BAF_{loc} = 2.2 for 0.3% TOC) when compared to a sediment with a higher TOC content (BAF_{loc} = 0.82 for 2% TOC), which may have been due to a higher rate of feeding caused by low TOC. Conversely, Meador et al. (1995) found BAF_{loc}s lower than expected for a polychaete (*Armandia brevis*) exposed to low TOC in field-contaminated sediments (0.3–0.5% TOC). This disparity may have been caused by a higher affinity of PAHs to sedimentary organic carbon at relatively low TOC concentrations and the possible lack of increased feeding by *A. brevis* in these sediments.

Occasionally the bioaccumulation factors are not as predicted, which may be explained by organism behavior. Varanasi et al. (1985) found large differences in day 7 TSRs between infaunal amphipods (*Eohaustorius washingtonianus* and *Rhepoxynius abronius*) and a deposit-feeding clam (*Macoma nasuta*) for benzo[a]pyrene, which may be indicative of uptake route or feeding habits. The amphipod produced higher TSRs than the clam even though their metabolism for this compound was much greater. The higher TSRs for the amphipods could have been caused by a higher rate of uptake leading to higher body burdens after 7 d or because the amphipods were living beneath the sediment surface and were exposed continually to interstitial water laden with benzo[a]pyrene. Conversely, the clam may have been frequently closed, reducing its uptake from ingested sediment and ventilated waterborne benzo[a]pyrene.

The reader should be cautious when interpreting bioaccumulation data because many studies are not conducted under equilibrium (sediment–water) or steady-state (animal–exposure medium) conditions. Additionally, many species are capable of substantial metabolism of PAHs which will severely bias the results.

D. Differential Accumulation

A few authors have noticed a pattern of differential accumulation that varies over major PAH groups (e.g., compounds containing 2 through 6 aromatic rings). Varanasi et al. (1985) reported that 2-, 3-, and 5-ring PAHs were poorly taken up by amphipods (*Eohaustorius washingtonianus* and *Rhepoxynius abronius*) and a clam (*Macoma nasuta*) when compared to 4-ring compounds. This pattern may have resulted from the volatility of the 2- and 3-ring compounds, which may be released directly without metabolism from the organism, slower uptake kinetics of the more hydrophobic PAHs, and the reduced uptake of the 5- and 6-ring compounds, which are suspected of being more tightly bound to organic carbon and hence less available to organisms. A differential pattern of uptake of PAHs was also found by Wade et al. (1988) for oysters. Their study showed that the 3- and 4-ring aromatic compounds produced the highest mean weight percentage of PAHs accumulated (approximately 40% each) while the 2- and 5-ring

compounds were both much lower, 15% and <5%, respectively (Fig. 15). A comparison with mean sediment concentrations showed enhanced tissue accumulation of the 2- and 3-ring compounds and depressed accumulation for the 4- and 5-ring compounds. In contrast, Foster and Wright (1988) found no pattern in bioaccumulation factors for PAHs in clams (*Macoma balthica)* or polychaetes (*Nereis succinea*) with number of aromatic rings.

Differential accumulation has also been observed in a vertebrate. Studies with an eel (*Anguilla anguilla*) collected from six sites in the Netherlands showed higher proportions of 2- and 3-ring PAHs and less of the 4- and 5-ring compounds in the eels, whereas in sediments the relative levels of these PAH classes were reversed (van der Oost et al. 1994). The reason for the differences in the proportions of the different ring classes between sediment and tissue is not clear and may be due to differences in bioavailability, rates of metabolic conversion, or differential persistence in the sediments. Varanasi and Gmur (1981) showed a much higher proportion of benzo[a]pyrene (five rings) as compared to naphthalene (two rings) as metabolites in English sole (*Parophrys vetulus*) exposed to sediment laden with

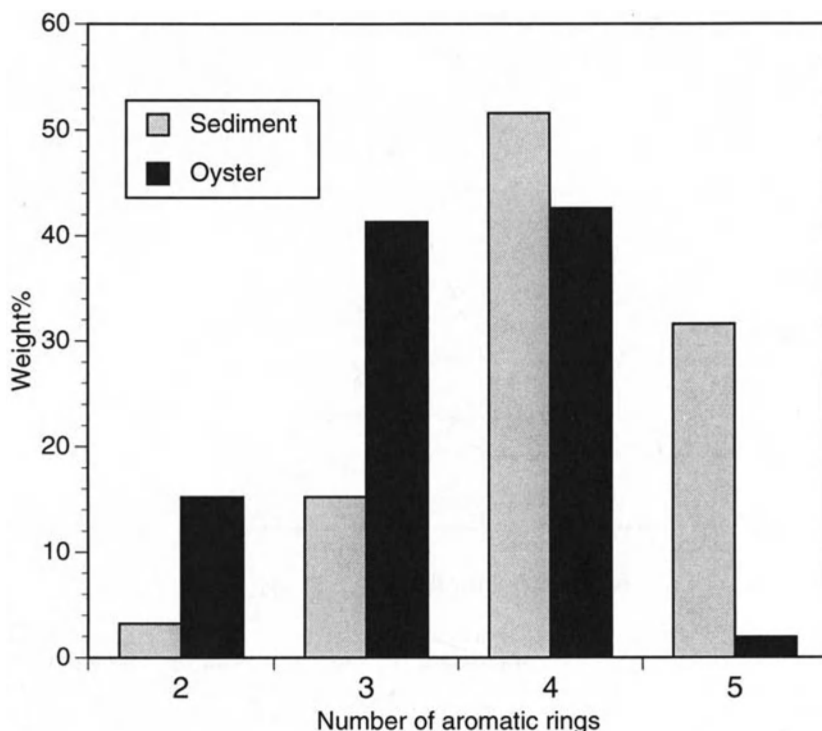

Fig. 15. Mean weight percentage of PAHs in sediment (*light bars*) and oyster tissue (*dark bars*) (*Crassostrea virginica*) categorized by number of aromatic rings. Samples are from the Gulf of Mexico. (Redrawn from Wade et al. 1988.)

PAHs. These findings suggest that the 4- and 5-ring structures may be metabolized to a greater extent in eels than the 2- and 3-ring structures, thus giving the appearance that 2- and 3-ring compounds may be more bioavailable.

We have examined the BAF_{loc}s for PAHs, which are hypothesized to be the same for each PAH, from another study (Meador et al. 1995) and have found an interesting pattern of PAH accumulation. The BAF_{loc}s for *R. abronius* exhibited the same pattern as that found by Varanasi et al. (1985) for this species, but a different pattern was found for the deposit-feeding polychaete (*Armandia brevis*) (Fig. 16) which is more similar to that shown for the deposit-feeding clam (*M. nasuta*) (Varanasi et al. 1985). The differences observed are likely the result of feeding mode. Because the amphipods in the study by Meador et al. (1995) were assumed to have inadequate dietary input, they probably received most of their tissue burden of both LPAHs and HPAHs through water uptake; hence, the BAF_{loc} would not be an appropriate measure of bioaccumulation. It has been proposed by Meador et al. (1995) that both deposit feeders and nondeposit

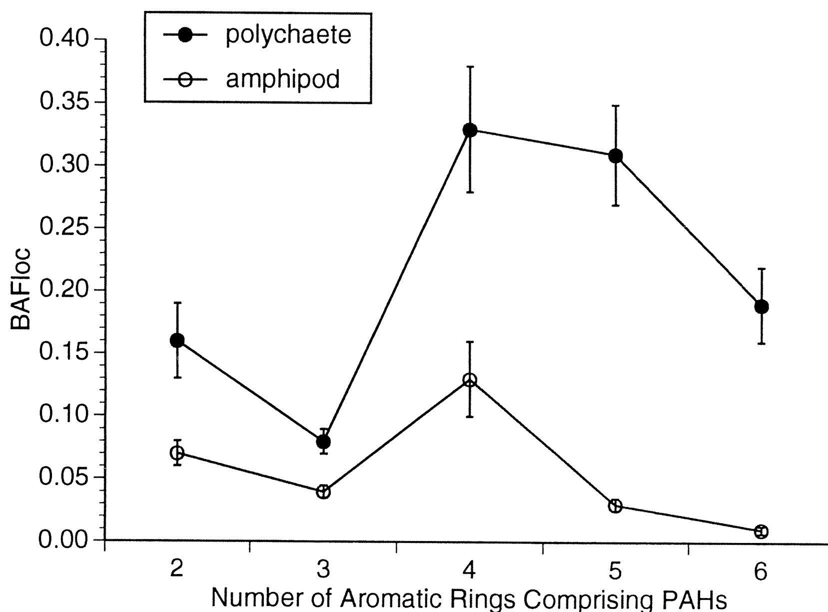

Fig. 16. Bioaccumulation factor (normalized to organism lipid and sediment organic carbon) as a function number of aromatic rings in PAHs. Mean and standard error of the mean BAF_{loc} for PAHs consists of 2–6 aromatic rings. Each mean represents several PAHs and sediments (sites: $n = 7$ for amphipod; $n = 5$ for polychaete). [See Table 1 for PAHs and ring categories.] Polychaete (*Armandia brevis*) and amphipod (*Rhepoxynius abronius*) individuals were exposed to Raritan-Hudson estuary (New York) sediments for 10 d. See Appendix for BAF_{loc} definition. (Data from Meador et al. 1995.)

feeders receive a large percentage of their body burden of 2- and 3-ring compounds (plus the 4-ringed compounds pyrene and fluoranthene) through interstitial water, regardless of feeding mode, which would produce a similar pattern of BAF_{loc}s in infaunal species. Because the unfed amphipods probably received all of their body burden of the 4-, 5-, and 6-ring compounds through porewater, and because of a reduction in the bioavailable fraction of these PAHs by DOC, accumulation was much reduced. Hence, the deposit feeder had greater exposure relative to the amphipod because it ingested sediment containing these HPAH compounds. As for the most hydrophobic compounds, many authors have noted that the decline in accumulation of 6-ring compounds as seen in both species may be caused by a decrease in uptake efficiency as hydrophobicity increases (Konemann and van Leeuwen 1980; McKim et al. 1985; Oliver 1984). Because this bioassay system did not include a dietary input for the amphipod, the results could be quite different for organisms from a field study. Many of the popular amphipod species used in sediment bioassay systems have an undefined feeding regimen in the laboratory; hence, caution in interpretation of bioaccumulation and toxicity results relative to that which occurs in the field is warranted.

Summary

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the marine environment, occurring at their highest environmental concentrations around urban centers. While they can occur naturally, the highest concentrations are mainly from human activities, and the primary sources are combustion products and petroleum. Two factors, lipid and organic carbon, control to a large extent the partitioning behavior of PAHs in sediment, water, and tissue; the more hydrophobic a compound, the greater the partitioning to these phases. These two factors, along with the octanol-water partition coefficient, are the best predictors of this partitioning and can be used to determine PAH behavior and its bioavailability in the environment. It is well known that the lipid of organisms contains the highest levels of hydrophobic compounds such as PAHs, and that organic carbon associated with sediment or dissolved in water can have the greatest influence on PAH bioavailability. Partitioning of combustion-derived PAHs between water and sediment may be much less than predicted, possibly because associations with particles are much stronger than expected. This reduced partitioning may produce erroneous results in predicting bioaccumulation where uptake from water is important.

Accumulation of PAHs occurs in all marine organisms; however, there is a wide range in tissue concentrations from variable environmental concentrations, level and time of exposure, and species ability to metabolize these compounds. PAHs generally partition into lipid-rich tissues, and their metabolites can be found in most tissues. In fish, liver and bile accumulate

the highest levels of parent PAH and metabolites; hence, these are the best tissues to analyze when determining PAH exposure. In invertebrates, the highest concentrations can be found in the internal organs, such as the hepatopancreas, and tissue concentrations appear to follow seasonal cycles, which may be related to variations in lipid content or spawning cycles.

The major route of uptake for PAHs has been debated for years. For the more water-soluble PAHs, it is believed that the main route of uptake is through ventilated water and that the more hydrophobic compounds are taken in mainly through ingestion of food or sediment. There are many variables, such as chemical hydrophobicity, uptake efficiency, feeding rate, and ventilatory volume, which may affect the outcome. The route of uptake may be an important issue for short-term events; however, under long-term exposure and equilibrium conditions between water, prey, and sediment, the route of uptake may be immaterial because the same tissue burdens will be achieved regardless of uptake routes. The key assumption of equilibrium between these different compartments may, however, rarely occur for PAHs.

Direct evidence for accumulation of PAHs by species that are able to metabolize these compounds is often difficult to obtain because of the rapid rate of biotransformation to metabolites that are not routinely detected by standard analytical techniques. If appreciable metabolism is occurring, then the bioaccumulation study needs to be performed using radiolabeled compounds to allow a mass-balance accounting of parent compounds and metabolites. Hence, for metabolically active species that accumulate very little parent compound, it may be more appropriate to monitor the metabolic products for evidence of accumulation. Two techniques have been developed to measure PAH metabolites; one utilizes HPLC fluorescence detection of metabolites in bile of teleosts and the other uses ^{32}P-postlabeling for DNA adducts that occur as a result of the interaction of metabolites and cellular DNA. Both techniques have displayed a highly positive correlation with environmental concentrations, making them useful for monitoring populations in our coastal areas.

Because their biotransformation tends to follow taxonomic lines, PAHs accumulate only in those invertebrates with low enzymatic activity toward these compounds (e.g., molluscs). Even within taxonomic groups, such as polychaetes and crustaceans, metabolic capability can be quite variable. In general, fish have a large capacity to biotransform PAHs while molluscs have a limited ability to metabolize them. Polychaetes and crustaceans are generally intermediate in their response, exhibiting at least an order of magnitude range in metabolic rate within these taxonomic groups.

In general, the majority of steady-state body burden appears to occur within days for the LPAHs and within a few weeks for the HPAHs. An examination of PAH half-lives indicates that time to 50% steady state occurs within a few days for all PAHs in fish and within a week or two for invertebrates, depending on the hydrophobicity of the PAH and the

metabolic capacity of the species. Steady state occurs rapidly in those species that can metabolize PAHs and more slowly in those that rely strictly on diffusive loss for elimination.

It has been suggested that PAHs may persist in organisms. A recurring theme in many studies indicates that organisms exposed to PAHs for a short time will completely eliminate their acquired burden when exposed to a clean environment, whereas species chronically exposed to these compounds tend to retain a portion of their acquired burden that is resistant to elimination by metabolism or passive diffusion. This is advantageous for animals exposed to PAHs in acute events (e.g., oil spills) but detrimental to those living in chronically contaminated environments. Consequently, combustion PAHs may occur more commonly in sediment and organisms due to chronic inputs, whereas petroleum hydrocarbons in sediment and tissues may be associated with acute events. Unfortunately, even those chronically contaminated sites that have been remediated may have populations of organisms with persistent PAHs.

Bioconcentration and bioaccumulation factors (BCFs and BAFs) are useful ratios that can indicate steady-state exposure and expected tissue burdens based on environmental concentrations. BCFs generally increase with chemical hydrophobicity in those species with weak or nonexistent metabolic systems for PAH biotransformation, whereas sediment-based accumulation factors at equilibrium predict no correlation to hydrophobicity. It is conceivable to have a pattern of declining BCFs and BAFs with increasing hydrophobicity in species that can rapidly metabolize all PAHs and have reduced uptake due to declining bioavailability. Expected BCFs based on laboratory-determined uptake and elimination constants (k_1 and k_2) can be useful for predicting steady-state tissue burdens in field-collected organisms because metabolic activation is included in their determination. Lipid- and organic carbon-normalized BAFs (BAF_{loc}s) are also very useful when attempting to determine equilibrium tissue concentrations. Whenever BAF_{loc}s are below the theoretical maximum, factors such as the biotransformation of parent compound, changing exposure concentration, or insufficient time for accumulation may be the cause. If metabolic capacity can be accounted for, then spatial or temporal factors can be explored. An interesting pattern of accumulation over major ring classes of PAHs (e.g., 2- through 6-ringed compounds) has been observed, which may be a consequence of the mode of feeding, bioavailability, and the environmental matrix used for normalization.

There are many studies on PAH bioaccumulation concerning freshwater organisms that are relevant to this review. Great strides have been made in the mechanisms of bioavailability, bioaccumulation, and toxicokinetics using both marine and freshwater organisms, which probably do not differ greatly in their responses. The reader should be reminded that these mechanisms know no boundary and that studies from each major aquatic environment are relevant. Future work on the subjects of uptake efficiency, the role

of qualitative and quantitative differences in organic carbon in determining bioavailability, assumed environmental equilibrium, trophic transfer of parent compounds and metabolites, predictable accumulation factors, intra- and interspecific toxicokinetic differences, and the variability in toxicokinetic parameters as a function of chemical hydrophobicity, environmental changes, and physiological factors will help greatly to enhance our understanding of the mechanisms of PAH accumulation in marine organisms.

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Appendix

The following formulas and definitions are used in this review:

BAF (bioaccumulation factor) = [Tissue]/[Sediment]

BAF_{loc} (lipid and sediment organic carbon (oc) normalized bioaccumulation factor = ([Tissue]/f_{lip})/[Sed_{oc}])

BCF (bioconcentration factor estimated with toxicokinetic constants) = k_1/k_2

BCF_{free} (bioconcentration factor with free PAH in water) = [Tissue]/[$Water_{free}$]

BCF_{pred} is BCF predicted. For example the equation from McCarty (1986); = $0.046\,K_{ow}$.

BCF_{total} (free and bound (= total) PAH in water) = [Tissue]/[$Water_{total}$]

DOC is dissolved organic carbon in water (usually in mg/L or ppm).

f_{lip} is the fraction of tissue that is lipid (usually in dry weight).

f_{oc} is the fraction of sediment that is organic carbon (usually in dry weight).

f_{doc} is the fraction of water that is dissolved organic carbon (usually (mg/L)(1 × 10^{-6})).

Half-life = time for half of the concentration of a xenobiotic to be eliminated from tissue = $0.693/k_2$ (in hr or d)

k_1 (uptake clearance constant) = $\frac{[\text{Tissue}]}{[\text{Water}]t}$ (units are ml g^{-1} $time^{-1}$)

k_2 (elimination rate constant) = $\left[\left(\ln \frac{[\text{Tissue}]_t}{[\text{Tissue}]_{t=o}}\right)/t\right]$ (units are $time^{-1}$)

K_{doc} (DOC–water partition coefficient) = [$Water_{bound}$]/([$Water_{free}$] * f_{doc})

$K_{oc,f}$ (sediment organic carbon–water partition coefficient for water concentration with free PAH) = ([Sediment]/f_{oc})/[$Water_{free}$]

$K_{oc,t}$ (sediment organic carbon–water partition coefficient for water concentration with free and bound PAH) = ([Sediment]/f_{oc})/[$Water_{total}$]

K_{ow} (octanol–water partition coefficient) = [Octanol]/[Water]

K_p (sediment–water partition coefficient) = [Sediment]/[Water]

LC_{50} is the concentration in the environmental matrix (water or sediment) that causes 50% of the individuals to die at a specific time interval.

LD_{50} is the dose or concentration in the tissues that causes 50% of the individuals to die at a specific time interval.

Sed_{oc} (organic carbon-normalized sediment concentration) = $[Sediment]/f_{oc}$

TOC is total organic carbon in sediment (usually in percent of sediment dry weight)

TSS_{50} (time for tissue concentration to reach 50% of steady state) = $0.693/k_2$

$[Water]_{free}$ (equilibrium concentration of free PAH in water) = $[Water_{total}]/(1 + f_{doc}*K_{doc})$

$[Water]_{total}$ (equilibrium water concentration of free and bound PAH) = $([Sediment]/f_{oc})/K_{oc}$

Formulas for uptake model:

ng taken up per day by ventilation
= (mL water ventilated d^{-1}) (ng PAH /mL) (uptake efficiency/100)

ng taken up per day by ingestion
= (g sediment ingested d^{-1}) (ng PAH /g) (uptake efficiency/100)

Percent taken up from water = amt from water/ (amt from water + amt from sed) × 100

PAH abbreviations: naph (naphthalene); 1methnaph (1-methylnaphthalene); 2methnaph (2-methylnaphthalene); biphen (biphenyl); acnaphyl (acenaphthylene); acnaphen (acenaphthalene); fluorn (fluorene); dimethnaph (dimethylnaphthalene); anthrcn (anthracene); phenanth (phenanthrene); methphen (1-methylphenanthrene); fluoran (fluoranthene); chrys (chrysene); methpyr (methylpyrene); b[a]fluorn (benzo[a]fluorene); b[b]fluorn (benzo[b]fluorene); b[a]anth (benz[a]anthracene); b[e]pyr (benzo[e]pyrene); b[a]pyr (benzo[a]pyrene); dibenzan (dibenz[a,h]anthracene); peryl (perylene); b(b)fluor (benzo(b)fluoranthene); b(k)fluor (benzo(k)-fluoranthene); b[ghi]fluor (benzo[ghi]fluoranthene); b[ghi]peryl (benzo-[ghi]perylene); b[iso]fluor (benzofluoranthene isomers); indpyr (indeno-[1,2,3-cd]pyrene).

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