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Mehran Alaee *Editor*

Dioxin and Related Compounds

Special Volume in Honor of Otto Hutzinger

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Dioxin and Related Compounds

Special Volume in Honor of Otto Hutzinger

Volume Editor: Mehran Alaee

With contributions by

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Aims and Scope

Since 1980, *The Handbook of Environmental Chemistry* has provided sound and solid knowledge about environmental topics from a chemical perspective. Presenting a wide spectrum of viewpoints and approaches, the series now covers topics such as local and global changes of natural environment and climate; anthropogenic impact on the environment; water, air and soil pollution; remediation and waste characterization; environmental contaminants; biogeochemistry; geoecology; chemical reactions and processes; chemical and biological transformations as well as physical transport of chemicals in the environment; or environmental modeling. A particular focus of the series lies on methodological advances in environmental analytical chemistry.

Series Preface

With remarkable vision, Prof. Otto Hutzinger initiated *The Handbook of Environmental Chemistry* in 1980 and became the founding Editor-in-Chief. At that time, environmental chemistry was an emerging field, aiming at a complete description of the Earth's environment, encompassing the physical, chemical, biological, and geological transformations of chemical substances occurring on a local as well as a global scale. Environmental chemistry was intended to provide an account of the impact of man's activities on the natural environment by describing observed changes.

While a considerable amount of knowledge has been accumulated over the last three decades, as reflected in the more than 70 volumes of *The Handbook of Environmental Chemistry,* there are still many scientific and policy challenges ahead due to the complexity and interdisciplinary nature of the field. The series will therefore continue to provide compilations of current knowledge. Contributions are written by leading experts with practical experience in their fields. *The Handbook of Environmental Chemistry* grows with the increases in our scientific understanding, and provides a valuable source not only for scientists but also for environmental managers and decision-makers. Today, the series covers a broad range of environmental topics from a chemical perspective, including methodological advances in environmental analytical chemistry.

In recent years, there has been a growing tendency to include subject matter of societal relevance in the broad view of environmental chemistry. Topics include life cycle analysis, environmental management, sustainable development, and socio-economic, legal and even political problems, among others. While these topics are of great importance for the development and acceptance of *The Handbook of Environmental Chemistry,* the publisher and Editors-in-Chief have decided to keep the handbook essentially a source of information on "hard sciences" with a particular emphasis on chemistry, but also covering biology, geology, hydrology and engineering as applied to environmental sciences.

The volumes of the series are written at an advanced level, addressing the needs of both researchers and graduate students, as well as of people outside the field of

"pure" chemistry, including those in industry, business, government, research establishments, and public interest groups. It would be very satisfying to see these volumes used as a basis for graduate courses in environmental chemistry. With its high standards of scientific quality and clarity, *The Handbook of Environmental Chemistry* provides a solid basis from which scientists can share their knowledge on the different aspects of environmental problems, presenting a wide spectrum of viewpoints and approaches.

The Handbook of Environmental Chemistry is available both in print and online via www.springerlink.com/content/110354/. Articles are published online as soon as they have been approved for publication. Authors, Volume Editors and Editors-in-Chief are rewarded by the broad acceptance of *The Handbook of Environmental Chemistry* by the scientific community, from whom suggestions for new topics to the Editors-in-Chief are always very welcome.

Damià Barceló
Andrey G. Kostianoy
Editors-in-Chief

Volume Preface

On September 22, 2012, the environmental science community lost an innovator, mentor, colleague and friend. Professor Otto Hutzinger had a very distinguished professional career and was a champion who made many significant scientific contributions that shaped our understanding of the environmental chemistry, toxicity and analysis of dioxins and related POPs.

Otto Hutzinger was born on March 14, 1933, in Vienna. Between 1947 and 1952, he studied chemistry at the Polytechnic for Chemical Industry and Trade (Vienna, Austria). Subsequently, he worked at EBEWE Pharma until he and his wife (Freda) immigrated to Canada in 1956. In Canada, he studied at the University of Saskatchewan in Saskatoon with Dr. Ronald Heacock and earned an MSc degree in 1963 and, two years later, a PhD degree in chemistry. In that same year, he became a Canadian citizen. Between 1965 and 1967, he was an NIH postdoctoral fellow at the University of California in Davis.

From 1967 to 1974, Otto returned to Canada accepting a position as a scientific research officer at the National Research Council of Canada, in Halifax, Nova Scotia. In 1974, he was appointed professor and director of the Laboratory of Environmental Toxicology and Chemistry at the University of Amsterdam. In 1983, Otto was appointed chair of ecological chemistry and geochemistry, University of Bayreuth, where he remained until his retirement in 1998.

Professor Hutzinger was a strong proponent of disseminating scientific information, and in 1980, he established the Dioxin Symposia, which have become the most prestigious of international conferences on persistent organic pollutants. He founded several very highly respected scientific journals including *Chemosphere*, *Environmental Science and Pollution Research* and *Toxicological and Environmental Chemistry*. In 1980, he became the founding editor-in-chief of *The Handbook of Environmental Chemistry*.

In appreciation of his significant contributions to the field of environmental chemistry and toxicology, Dr. D. Barcelò, editor-in-chief, and Dr. T. Wassermann, publishing editor of *The Handbook of Environmental Chemistry*, have dedicated a

special volume of the handbook as a tribute to his outstanding career. I have the honour of editing this special volume.

Professor Hutzinger was an innovator and champion in the field; hence it is a fitting tribute to dedicate this volume of *The Handbook of Environmental Chemistry* on recent advances in the environmental chemistry of dioxins and related POPs to him. This volume is a collection of manuscripts written by individuals who have been inspired by his pioneering work and have continued in his footsteps by refining our current understanding in this field and opening new research areas. This volume consists of 14 chapters. In the first chapter, Heidelore Fiedler identifies sources of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. In a subsequent chapter, Shinichi Sakai from Kyoto University describes the continued challenge for environmental control of polychlorinated dibenzo-dioxins and furans, polychlorinated biphenyls and brominated flame retardants. Analysis of dioxins and dioxin-like compounds, by far the most challenging and expensive technique in the determination of persistent organic pollutants in environmental matrices, is covered by Eric Reiner. In another chapter, Peter Fürst and his colleagues describe a cost-effective alternative method for determination of dioxins in food and feed based on gas chromatography combined with triple quadrupole mass spectrometry. Additional chapters deal with environmental levels and trends of dioxins and related compounds in environmental compartments. Wenning and Martello give an overview of levels and trends in the abiotic matrices. In the following chapter, She et al. discuss the occurrence of these compounds in aquatic environment. Shane de Solla discusses the exposure, bioaccumulation, metabolism and monitoring of persistent organic pollutants in terrestrial wildlife. A chapter on food and feed, the major routes for human exposure, is presented by Martin Rose; levels and trends of polychlorinated dibenzo dioxins and dioxins and furans in humans are discussed by Hernandez Welden and LaKind in the next chapter. Kunise and Tanabe combine different environmental compartments by discussing the occurrence of contaminants in Asian countries. The quintessential question in all of these investigations is "What are the health risks to humans and the environment". This subject is discussed by Martin Rose in the chapter "Risk Assessment for Dioxins and Related Compounds". The following three chapters focus on specific groups of compounds. DDT and its metabolites are discussed by Mirmigkou and de Boer, which was the first group of compounds that caught the attention of the environmental community and started the environmental movement. The second group of compounds is flame retardants, some of which were identified by Professor Hutzinger in the 1980s as potential contaminants of concern. Covaci and Malarvannan discussed determination, occurrence and trends of brominated flame retardants, and Marvin et al. discuss the analysis of chlorinated and phosphorus flame retardants.

Otto Hutzinger was an outstanding scientist whose work has significantly advanced the science of persistent toxic chemicals. It was always extremely important to Otto that new information is passed on to others through scientific meetings and publications. This volume is tribute to his efforts to pass on valuable information to others.

Burlington, ON, Canada Mehran Alaee
April 2016

Contents

Release Inventories of Polychlorinated Dibenzo-*p*-Dioxins and Polychlorinated Dibenzofurans

Heidelore Fiedler

Abstract For unintentionally generated persistent organic pollutants such as polychlorinated dibenzo-*para*-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), the development and maintenance of national release inventories is an obligation for parties to the Stockholm Convention on Persistent Organic Pollutants. About 20 years after the first dioxin inventories have been published, a systematic approach has been developed and now is applied worldwide to establish complete, comparable inventories that are consistent in format and content. The basis for such inventories is the "Toolkit," a collection of emission factors and description of activities and processes that form and release PCDD/PCDF. The Toolkit uses a five-vector approach, i.e., not only releases to air but also to other compartments such as water, land, product, and residue are included. The assessment of the quantitative data for releases from ten source groups to five release vectors provides interesting insight in the country's geographic, economic, and development status. After the first round of reporting PCDD/PCDF inventories, 86 inventories have been assessed, and it can be seen that the total releases of PCDD/PCDF from the ten source categories have a positive correlation with the size of the population and a negative correlation with economic status.

Keywords Economic status, Polychlorinated dibenzofurans, Polychlorinated dibenzo-*p*-dioxins, Regional assessment, Release inventory, Stockholm Convention

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Contents

Abbreviations

µg	Microgramme
CEE	Central and Eastern European (group of countries)
COP	Conference of the Parties (to the Stockholm Convention)
EF	Emission factor
g	Gramme
GNI	Gross national income
GRULAC	Group of Latin American and Caribbean Countries
HCB	Hexachlorobenzene
ISO	International Organization for Standardization
kg	Kilogramme
km^2	Square kilometer
NIP	National implementation plan
p	*para*
PCB	Polychlorinated biphenyls
PCBz	Pentachlorobenzene
PCDD	Polychlorinated dibenzo-*para*-dioxins
PCDF	Polychlorinated dibenzofurans
pg	Picogramme
POPs	Persistent organic pollutants
PPP	Purchasing power parity
SG	Source group
TEF	Toxicity equivalency factor
TEQ	Toxic equivalent
UNEP	United Nations Environment Programme
WEOG	Western European and Others Group
WHO	World Health Organization
yr	Year

1 Introduction

Polychlorinated dibenzo-*para*-dioxins and polychlorinated dibenzofurans (PCDD/PCDF) have never been produced for any purpose other than laboratory experiments. They are unintentionally formed in industrial-chemical processes [1–3], such as chemical manufacture or pulp and paper production, and thermal processes [4–6], such as waste incineration, recycling of metals, the production of minerals or forest fires, and release to the environment. The predominant mechanism or pathway to generate PCDD/PCDF can vary from process to process resulting in a wide range of source-specific emission factors that also take into account different factors, such as reduction and abatement technologies, to reduce the releases [7]. It is generally accepted that the main sources of PCDD/PCDF are human activities [8].

The Stockholm Convention on Persistent Organic Pollutants (POPs) entered into force on 17 May 2004. In August 2015, 179 countries were party to the Convention through ratification, acceptance, approval, or accession [9]. An updated list of parties to the Convention and full information on the Stockholm Convention is available on the Web site of the Treaty Section of the United Nations at the following URL address: http://untreaty.un.org. The Convention intends to stop production and use of intentional POPs (e.g., pesticides such as DDT or industrial chemicals such as polychlorinated biphenyls (PCB)) and "reduce the total releases derived from anthropogenic sources" of unintentional POPs (i.e., polychlorinated dibenzo-*para*-dioxins/polychlorinated dibenzofurans (PCDD/PCDF), hexachlorobenzene (HCB), polychlorinated biphenyls (PCB), and pentachlorobenzene (PCBz)). In order to track the environmental and human exposure to these compounds, the Convention requires the parties to undertake continued measurements of their releases and concentrations. Article 5 of the Stockholm Convention [10] requests countries to develop an action plan "designed to identify, characterize and address the release of the chemicals" regulated by the Convention. The action plan shall include "[...] an evaluation of current and projected releases, including the development and maintenance of source inventories and release estimates."

In order to assist countries to establish release inventories of polychlorinated dibenzo-*p*-dioxins and dibenzofurans at national or regional level and to fulfill the requirements on release reduction under Article 5 of the Convention, UNEP through an expert group has developed a Toolkit for the development of release inventories of unintentional POPs [11]. The information contained therein comes from published scientific literature, government reports, Internet sources and through personal communication to the UNEP expert group. The Toolkit (published in 2013) is the most comprehensive available compilation of emission factors for all relevant PCDD/PCDF sources and is useful particularly in countries where measurement data are limited, enabling the elaboration of source inventories and release estimates by using the default emission factors. It is also useful in countries where national measurement data are available, as a reference document for data comparison and validation purposes. Therefore, the Conference of the Parties at its fifth meeting in 2011 (COP-5) encouraged the use of the Toolkit and adopted the

reporting format from the Toolkit. Through its structure for reporting, i.e., ten source groups and five release vectors, it is possible to gain some further insight into the global situation as to the sources of PCDD/PCDF releases [12].

This paper gives a brief overview on PCDD/PCDF release inventories as of December 2014 and then presents assessments of national inventories according to the total releases and specific releases of PCDD/PCDF, such as according to source groups or release vectors. Further, statistical assessments are presented in particular in relation with geographic and socioeconomic factors.

2 History

Sources of PCDD/PCDF have been addressed systematically since about 1980 when Esposito et al. [13] published the first comprehensive report on sources of "dioxins," especially tetrachlorodibenzodioxin (TCDD). Since about 25 years, dioxin inventories have been presented. For other persistent organic pollutants, attempts have been undertaken to quantify their releases and regional distribution such as by Breivik et al. [14]; however, the abundance of information as exists for PCDD/PCDF is not available for any other POP.

At Dioxin'90 [15], Fiedler and coworkers [16] published the paper entitled "Dioxin Emissions to the Air: Mass Balance for Germany Today and in the Year 2000." The results were mainly based on measured emission data and had a total annual emission of 928.5 g I-TEQ to air from sources in Germany (former Federal Republic of Germany). Although releases in solid residues such as slags, fly ashes, and sludges have been quantified, a systematic approach for estimating these releases had not been undertaken.

In 1999, UNEP published a report presenting the results of 15 emission inventories; the reference year was around 1995 [17]. Figure 1 shows the distribution of sources within and between countries.

With the entry into force of the Stockholm Convention and the recommendation to use one methodology for the development and presentation of national PCDD/PCDF inventories and report results back to the Conference of the Parties, comparison of release inventories became easier.

The Toolkit uses the five-vector approach and countries are able to estimate PCDD/PCDF releases from each source category to the following environmental media: air, water (surface and ground water, including marine and estuarine water), and land (surface soils) as well as to these process outputs: products (such as chemical formulations, including pesticides or consumer goods such as paper, textiles, etc.) and residues (including certain liquid wastes, sludge, and solid residues, which are handled and disposed of as waste or may be recycled).

In the early 2000s and presented at Dioxin 2004 in Berlin, 23 national release inventories were available that have been made with the UNEP Toolkit methodology [18]. Among the most important sources, open fires in agriculture/forests as well as open burning of wastes have been identified as the major sources of PCDD/

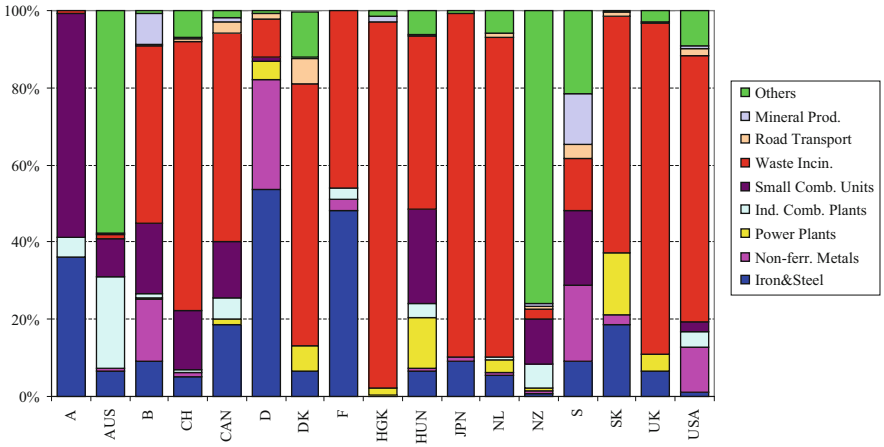


Fig. 1 Contribution of PCDD/PCDF emission source groups per country (% on the basis of TEQ yr^{-1}) [17]

PCDF. At Dioxin2007, Fiedler [19] presented the actual status of global POPs inventories, which were divided into two types of PCDD/PCDF inventories:

1. Estimated releases of PCDD/PCDF to air and as totals by countries that did not apply the UNEP Toolkit methodology (12 countries): Air emissions were 3,804 g TEQ yr^{-1} and total releases were 4,148 g TEQ yr^{-1}. Without a common methodology, most country estimates reported emissions to air only, did not address the same sources, and typically did not assess releases to water, land, residues, or products.
2. Estimated releases by countries that applied the UNEP Toolkit methodology (43 countries; different from countries included in (1) above): Air emissions were 10,911 g TEQ yr^{-1} and total releases 23,877 g TEQ yr^{-1}. Since these inventories used the same methodology, typically all sources listed in the Toolkit were assessed and the five release vectors included. Therefore, the results between countries are better comparable.

In this paper [19], for the first time, so-called population equivalents in μg TEQ per year were included. Such normalization was found to be helpful to put results into perspective and which can also serve as an orientation for a country if the own estimate fits into the scale of estimates from other countries. Across all countries, it was found that the following average releases per capita and per year did apply: 12 μg TEQ yr^{-1} $person^{-1}$ to air and 21 μg TEQ yr^{-1} $person^{-1}$ for total releases.

At Dioxin 2012, Fiedler et al. [20] presented a geographic and socioeconomic assessment of PCDD/PCDF inventories, based on 68 national release inventories; the majority of them were developed using the 2005 version of the UNEP Standardized Toolkit. The total releases accounted for 58,700 g TEQ per year.

Based on the same dataset, the quantitative releases have been correlated to geographic, demographic, and source-specific information, exploring the release

patterns of PCDD/PCDF influenced by economic status and methodology that is fair, accurate, and objective enough to assess International PCDD/PCDF Reduction Burden [21].

In this research, the results of national release inventories of PCDD/PCDF that have been developed by using the UNEP Toolkit are presented and evaluated. Using the same methodology, the Toolkit, it is ensured that the source inventories and release estimates are complete in the sense of the Toolkit methodology, transparent, and consistent in format and content. The results will allow parties and others to compare results, identify priorities, mark progress, and follow changes over time at the national, regional, and global levels.

3 Materials and Methods for the Assessment of Dioxin/Furan Inventories

3.1 Inventory Methodology

The "Toolkit for Identification and Quantification of Releases of Dioxins, Furans and Other Unintentional POPs under Article 5 of the Stockholm Convention" [11] or "PCDD/PCDF Toolkit" for short provides emission factors for the five release vectors, i.e., air (EF_{Air}), water (EF_{Water}), land (EF_{Land}), product ($EF_{Product}$), and residue ($EF_{Residue}$). Together with national activity data, this approach allows the development of release inventories for total releases (all source groups and all five release vectors) but also presentation of releases to, e.g., air only or sectoral consideration according to source groups.

Release inventories are obtained by measuring the "activity rate" of dioxin-releasing activities and multiplying them by a specific "emissions factor." For a given country, the total releases are given by

$$\mathrm{TEQ_{PCDD\text{-}PCDF}} = \sum \mathrm{j\ ActivityRate}_j \times \mathrm{EmissionFactor}_j$$

where $\mathrm{ActivityRate}_j$ is the activity rate of the source j and the $\mathrm{EmissionFactor_j}$ is the emission factor for this source j for each of the five vectors, including air, water, land, product, and residue.

For this assessment, the national inventories, compiled according to the UNEP Toolkit methodology for estimating PCDD/PCDF releases into the environment with five vectors and ten source groups, have been entered into an MS Excel databank and assessed further.

Values of PCDD/PCDF releases are presented as toxic equivalent (TEQ) using the concept of toxic equivalency which measures the relative dioxin-like toxic activity of different congeners of polychlorinated dibenzo-*p*-dioxins and dibenzofurans and expresses the result in a single number, the toxic equivalent (TEQ). The Stockholm Convention on Persistent Organic Pollutants initially uses the toxicity

equivalency factors (TEFs) established by a World Health Organization (WHO) expert group in 1997 and published in 1998 [22] and not yet the scheme established in 2005 [23]. The TEFs rank the toxicity of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in comparison with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Following the UNEP Toolkit methodology, the TEQ is not adhered to a specific scheme of toxicity equivalency factors (TEFs). For their "order-of-magnitude" estimates of emission factors, the differences between the WHO_{1998}-TEFs and other TEF schemes previously or later established are negligible. Therefore, the TEF scheme accompanying the emission factors is not detailed further in the Toolkit, and as a consequence, the national releases are expressed in g TEQ per year without further specification of the TEF scheme.

3.2 Data Sources

Early PCDD/PCDF inventories have been compiled from the published literature and national reports. With the entry into force of the Stockholm Convention on Persistent Organic Pollutants, official national reporting has gained importance and information has been drawn from, e.g., submissions of parties to the Stockholm Convention according to national reporting under Article 15 or from national implementation plans prepared according to Article 7. Statistical data on population, economics, and major pollutant emissions have been extracted from the World Bank database .

In order to obtain comparable results for assessment, PCDD/PCDF inventories have been compiled from reports submitted by parties to the Stockholm Convention in their national implementation plans (NIPs) [24], national reporting formats according to Article 15 [25], other national reports or the scientific literature. Within its program of work, the Chemicals Branch of the United Nations Environment Programme (UNEP) regularly searches and updates a database, which is maintained in MS Excel. Inventory information is compiled according to ten source groups covering the main sources of PCDD/PCDF and five release vectors, i.e., air, water, land, product, and residue.

The statistical data such as population, land area, and gross national income per capita were extracted from the World DataBank, compiled and published by the World Bank [26]. For denominators such as population and GNI, these informations were referred to according to the reference year of the PCDD/PCDF inventory. Economies are classified according to GNI per capita (gross national income per capita) using the World Bank Atlas method. The four economic groups are (http://data.worldbank.org/about/country-classifications; economies are divided according to 2012 GNI per capita, calculated using the World Bank Atlas method) as follows: low income (L), lower middle income (LM), upper middle income (UM), and high income (H). It should be noted that both denominators were found to be highly volatile; e.g., whereas countries belonging to the low-income group in 1999 had a GNI PPP^{-1} lower than 755 international dollar, the threshold in 2011 was at 1,205 international dollar

(http://econ.worldbank.org/WBSITE/EXTERNAL/DATASTATISTICS/0,,contentMDK:20487070~menuPK:64133156~pagePK:64133150~piPK:64133175~theSitePK:239419,00.html).

4 Results

4.1 Overall Results

In 2014 and used in this review, 85 national inventories and one inventory for Hong Kong, Special Administrative Region (CHN-HKG SAR), have been available for assessment (Table 1). For all countries, the most recent inventory has been used. It should be noted that the reference years for which the releases have been estimated range over 12 years with the inventory for the Philippines being the oldest (reference year 1999) and for Zimbabwe being the most recent one (reference year 2011). Of the 86 inventories, seven countries reported releases to air only (AUT, BGR, CHE, DEU, FIN, FRA, RUS).

Figure 2 shows the graphical sketch for each country in Table 1 for the total releases and releases per vector; the releases summed-up per release vector are shown in Table 2. Countries colored in green have releases much lower than the average of all countries, and countries colored in red have releases much higher than the average (for both 5% or 95%, resp.). With respect to total releases, there are 17 countries with total releases significantly lower than the average of all countries and 11 countries that have significantly higher emissions (shown in red color). Countries releasing ±10% of the calculated average are shown in yellow color.

4.2 Quantitative Inventory Results Using Country Basis

4.2.1 Releases According to Five-Vector Approach

The total releases accounted for 70,819 g toxicity equivalents per year (g TEQ yr^{-1}). Of these, 47% were emitted to air (approximately 33,500 g TEQ yr^{-1}), 32% were found in residues (approximately 22,600 g TEQ yr^{-1}), 11% released to land (approximately 7,700 g TEQ yr^{-1}), and smaller amounts, i.e., 8%, were attributed to products (approximately 5,700 g TEQ yr^{-1}) and only 2% to water (approximately 1,300 g TEQ yr^{-1}) (Table 2). It should be noted that the ashes generated in open burning processes such as forest or agricultural fires or from open burning of waste were assigned as "release to land." Numerically, the highest releases were from China (10,238 g TEQ yr^{-1}) followed by India (8,658 g TEQ yr^{-1}) (among others, colored red in Fig. 2); the lowest releases were reported for Niue (0.56 g TEQ yr^{-1}) followed by Brunei Darussalam and Samoa (1.4 g TEQ yr^{-1}).

Table 1 National releases of PCDD/PCDF per country and release vector ($n = 84$)

Country	ISO-3	Air	Water	Land	Product	Residue	Total	Reference year
Albania	ALB	58.7	57.5	0.007	26.8	0.07	143	2004
Argentina	ARG	874	3.1	241	29.4	964	2,111	2003
Armenia	ARM	5.49	5.27	0.83	17.5	22.9	52	2001
Australia	AUS	498	3.41	1,278			1,780	2002
Austria	AUT	39.8					40	2004
Azerbaijan	AZE	67.7	9.22	1.00	1.92	47.9	128	2003
Bangladesh	BGD	188.4	62.1	0.0	198.0	37.3	486	2005
Belarus	BLR	26.1	0.46	1.71	0.05	73.1	101	2006
Benin	BEN	182	12.8	7.50	65.3	111	379	2002
Brazil	BRA	1,168	22.6	79.3	419	546	2,235	2008
Brunei	BRN	0.75	0.02	0.03	0.04	0.56	1.4	2001
Bulgaria	BGR	255					255	2003
Burkina Faso	BFA	300	12.6	59.7	10.7	402	785	2002
Burundi	BDI	190	0.10	0.00	0.05	4.91	195	2004
Cambodia	KHM	273	0	14.6	0	319	607	2004
Cameroon	CMR	397	0.02	186	0.03	13.7	597	2009
Canada	CDN	157	0.20	6.90			164	2004
Cape Verde	CPV	18.5	0.0004	0.001	0.00003	0.16	19	2005
Chile	CHL	51.7	2.55	16.9	7.17	7.31	86	2003
China	CHN	5,043	41.2	953	174	4,026	10,238	2004
China Hongkong SAR	CHN-HKG	2.70	0.86	0.48	0.06	16.7	20.8	2003
Colombia	COL	479	20.0	18.4	32.8	240	790	2002
Côte d'Ivoire	CIV	416		6.00		9.8	432	2002
Croatia	HRV	116		1.70	0.80	49.5	168	2001
Cuba	CUB	163	1.50	27.8	25.2	7.69	225	2002
Djibouti	DJI	50.8	55.6			12.9	119	2003
Ecuador	ECU	65.0	3.43	9.30	3.32	16.5	98	2002
Estonia	EST	13.7	0.15	0.12		15.2	29	2000
Ethiopia	ETH	154	3.84	5.95	29.6	21.5	215	2003
Fiji	FJI	11.2	0.12	0	7.31	0.60	19	2002
Finland	FIN	26.2					26	2005
France	FRA	247					247	2003
Gabon	GAB	135	5.27			32.8	173	2005
Gambia	GMB	107	3.96			65.4	177	2000
Germany	DEU	116					116	2002
Ghana	GHA	386	0.12	279	0	3.04	668	2004
India	IND	2,827	22.7	30.3	314	5,464	8,658	2009
Indonesia	IDN	1,847	81.2	436	3,545	1,443	7,352	2003
Iran	IRN	1,071	0.10	31.8	399	66.6	1,568	2005
Jordan	JOR	64.3	0.42	0.07	0.35	16.4	82	2003

(continued)

Table 1 (continued)

Country	ISO-3	Air	Water	Land	Product	Residue	Total	Reference year
Kenya	KEN	3,103	2.97	2.29	17	1,613	4,738	2005
Lao PDR	LAO	46.9	0.02	18.8		38.0	104	2005
Lebanon	LBN	79.0	1.20	0.02	3.05	82.6	166	2004
Liberia	LBR	186		7.50		121	315	2004
Lithuania	LTU	37.4	0.10	0.30	0.44	18.6	57	2005
Macedonia	MKD	163	3.14			8.61	175	2001
Madagascar	MDG	119	1.00	29.6	4.67	180	334	2002
Mali	MLI	35.0		0.53	2.17	1.79	39	2005
Mauritius	MUS	19.6	5.41	0.32	0.50	4.58	30	2003
Moldova	MDA	13.5	755	4.21		2.85	776	2001
Morocco	MAR	167	3.30	0.22	19.1	45.95	236	2003
Nepal	NPL	202	0.05	43	6.90	80.0	332	2003
New Zealand	NZL	17.4	1.58	35.5		34.9	89	2008
Nicaragua	NIC	191	0.47	303		3.15	498	2004
Nigeria	NGA	2,784	0.03	2,521		34.4	5,340	2004
Niue	NIU	0.39		0.004		0.17	0.56	2004
Norway	NOR	32.9	0.30	2.00		0.00	35	2004
Palau	PLW	0.10	0.00	0.00	0.00	2.14	2.25	2007
Panama	PAN	48.2	0.38	13.9	0.01	37.2	100	2005
Paraguay	PRY	70.7	0.20	8.50	0.22	76.3	156	2002
Peru	PER	193	0.16	61.5	4.14	165	424	2003
Philippines	PHL	328	43.8	46.9	77.6	38.1	534	1999
Portugal	POR	38.5	0.36	7.55	8.78	40.1	95	2006
Romania	ROU	136	0.15			454	590	2004
Russia	RUS	1,785					1,785	2007
Samoa	WSM	1.05				0.33	1.4	2004
Serbia	SRB	123	34.8	30.1	0.70	209	398	2006
Seychelles	SYC	4.06			0.07	1.27	5.4	2003
Slovenia	SVN	6.19	0.93		3.31	20.0	30	2005
South Africa	RSA	709	2.70	64.2	30.9	1,956	2,763	2006
Sri Lanka	LKA	171	0.08		6.45	79.5	258	2002
Sudan	SDN	376		52.4	24.0	540	992	2004
Swaziland	SWZ	47.3	0.01	69.5	0.13	0.23	117	2006
Switzerland	CHE	16.7					17	2005
Syria	SYR	352		208	61.9	0.88	623	2006
Tajikistan	TJK	32.0				141	173	2003
Tanzania	TZA	528	0	184	0	252	964	2007
Thailand	THA	286	1.33	6.64	8.36	767	1,070	2005
Togo	TGO	432	0.12	14.0		72.6	519	2002
Tunisia	TUN	139	0.50	0.66	0.97	67.2	209	2004
Turkey	TUR	1,249	0.30	96.0	123	695	2,163	2006
Uruguay	URY	18.7	0.15	1.24		26.8	47	2003
Venezuela	VEN	618	1.72	37.6	8.11	435	1,100	2007

(continued)

Table 1 (continued)

Country	ISO-3	Air	Water	Land	Product	Residue	Total	Reference year
Vietnam	VNM	16.0	1.46	1.05	2.19	48.2	69	2002
Zambia	ZMB	290		48.4		145	483	2004
Zimbabwe	ZWE	154	0.27	131		1.03	285	2011
Total		33,459	1,296	7,746	5,722	22,595	70,819	

Concentrations in g TEQ per year. Empty cells indicate that releases were either not estimated or do not occur (e.g., no emission factor in the Toolkit)

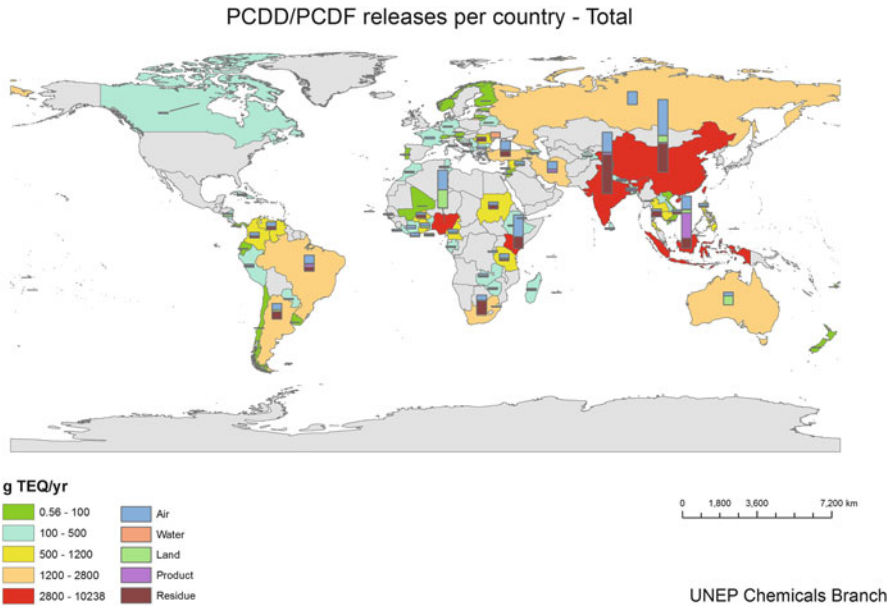


Fig. 2 Countries that reported low (*green* or *blue* color, resp.) or high releases of PCDD/PCDF (*orange* and *red* color, resp.). For countries colored in *grey*, no information has been provided so far; they are not included in Table 1. Countries with releases ±10% around the average calculated release are shown in *yellow* color. The *bar graphs* demonstrate the share for each release vector

Table 2 Summary of PCDD/PCDF releases per vector (based on 86 reports)

	Air	Water	Land	Product	Residue	Total
Release per vector (g TEQ yr^{-1})	33,459	1,296	7,746	5,722	22,595	70,819
Release per vector (% of total)	47	2	11	8	32	100

The 85 assessed countries (without CHN-HKG or Hong Kong SAR) constitute 44% from the total of 193 member states in the United Nations [27] but represent 68% of the global population of 6.966 billion in 2011 [28] (the reference year of the most recent inventory).

4.2.2 Regional Distribution of Releases and Socioeconomic Factors

Often, and as a recommended approach in the Stockholm Convention, release inventories may be developed on a regional basis. The total releases as well as the releases to the five release vectors are shown in Table 3. Accordingly, the highest overall releases are reported to originate in Asia (49%) followed by Africa (30%) and GRULAC (11%); CEE (6.4%) and WEOG (3.7%) together account for ~10% of the total releases. However, it should be noted that from the Asian and the African regions, 25 and 26 inventories have been reported, respectively, whereas from the other regions, the number of available inventories was lower (CEE = 13, GRULAC = 12, WEOG = 10). Further, the majority of the people "covered" by these inventories were from Asia (3.34 billion people or 71% of the population covered by these inventories), whereas from Africa, a population of "only" 560 million (0.56 billion or 12% of the population covered by these inventories) is included (Table 4).

Table 3 Summary of PCDD/PCDF releases per vector and per UN region (based on 86 reports)

UN region	No	Total (g TEQ yr^{-1})	Air (g TEQ yr^{-1})	Water (g TEQ yr^{-1})	Land (g TEQ yr^{-1})	Product (g TEQ yr^{-1})	Residue (g TEQ yr^{-1})
Africa	26	21,122	11,425	111	3,670	205	5,712
Asia	25	34,659	14,165	266	1,888	4,929	13,411
CEE	13	4,559	2,739	858	39	50	873
GRULAC	12	7,869	3,941	56	819	529	2,524
WEOG	10	2,610	1,190	5.8	1,330	8.8	75
Grand total	86	70,819	33,459	1,296	7,746	5,722	22,595
		100%	47%	2%	11%	8%	32%

Table 4 Number of countries reporting PCDD/PCDF releases per region and population covered (based on 86 reports)

Row labels	Number of inventories	Population covered by region in million inhabitants
Africa	26	560
Asia	25	3,344
CEE	13	212
GRULAC	12	383
WEOG	10	237
Grand total	86	4,736

Table 5 Summary of country statistic data used for assessment (based on 86 reports)

	Population (million)	Area (km^2)	GNI PPP $capita^{-1}$ (international dollar)
Minimum	0.0022	260	200
Maximum	1,296	17,098,240	43,740
Average	55	999,458	9,107
Total of all 86 countries	4,736	85,953,345	

Table 6 Summary of PCDD/PCDF releases per denominator and year (based on 86 reports)

	Total releases per denominator and year			Releases to air per denominator and year		
	mg TEQ $capita^{-1}$	mg TEQ km^{-2}	mg TEQ 1000 USD GNI PPP^{-1}	mg TEQ $capita^{-1}$	mg TEQ km^{-2}	mg TEQ 1,000 USD GNI PPP^{-1}
Minimum	0.87	18	0.032	0.20	17	0.006
Maximum	256	22,917	3,560	178	9,603	2,315
Average	39	3,004	340	20	1,480	169

The assessed countries exhibit a large range of characteristics with respect to population, area of the country, or gross national income per capita (Table 5). For example, the population of countries ranges over six orders of magnitude and has the smallest population in Niue (2,200 people in the year 2004) and the largest population in the People's Republic of China (1,30 billion in 2007). With an area of 260 km^2, Niue is also the smallest country; the largest country is the Russian Federation with an area of more than 17 million square kilometer. The poorest country within this dataset and according to the World Bank's statistics expressed as PPP is Liberia with a gross national income (GNI) of 200 international dollar per capita in 2004 and the economically strongest country is Brunei Darussalam with 43,740 international dollar in 2001, followed by Norway with 42,550 international dollar in 2004; the average GNI PPP per capita is 9,107 international dollar (for the countries included in this assessment and the corresponding reference year).

The minimum, maximum, and average releases of PCDD/PCDF for all release vectors ($PCDD/PCDF_{total}$) and to air are summarized in Table 6. The average total release per year (39 mg TEQ yr^{-1}) is about twice as high as the release to air only (20 mg TEQ yr^{-1}) underpinning the importance of the vector to air (paired with the fact that some countries estimated releases to air only; see Fig. 2).

According to income category following the World Bank classification (http://data.worldbank.org/about/country-classifications; economies are divided according to 2012 GNI per capita, calculated using the World Bank Atlas method), the number of countries reporting PCDD/PCDF releases within each income category

Table 7 Overview of annual releases to five release vectors according to income category (86 inventories)

Income category	# Countries	Air	Water	Land	Product	Residue	Total
		(g TEQ yr^{-1})					
High income	22	5,238	14	1,511	177	1,341	8,281
Upper middle income	31	11,873	217	1,801	1,223	9,933	25,043
Lower middle income	28	15,545	1,060	4,261	4,287	10,993	36,147
Low income	5	803	5	174	34	328	1,344
Total	86	33,459	1,296	7,746	5,722	22,595	70,819

varies: The annual PCDD/PCDF releases per release vector within each income category (L, LM, UM, H) are shown in Table 7. The highest number of countries belong to the economic group of upper middle income ($n = 31$), followed by the lower middle income ($n = 28$); the two extreme income groups have the lowest representation with five and 22 countries in the low-income and the high-income categories, respectively.

Annual PCDD/PCDF release of the 86 countries/regions ranged from 0.56 (Niue, UM) to 10,237 g TEQ yr^{-1} (China, UM) (Tables 1 and 8). The annual releases of the five largest emitters were from five upper middle-income countries (China = 10,238 g TEQ yr^{-1}; India = 8,658 g TEQ yr^{-1}; Indonesia = 7,352 g TEQ yr^{-1}; Nigeria = 5,340 g TEQ yr^{-1}; and Kenya = 4,738 g TEQ yr^{-1}), whereas the smallest releases were from two upper middle-income countries (Niue = 0.6 g TEQ yr^{-1} and Palau = 2.25 g TEQ yr^{-1}), one lower middle-income country (Samoa = 1.4 g TEQ yr^{-1}), and two high-income countries (Brunei Darussalam = 1.4 g TEQ yr^{-1} and Seychelles = 5.4 g TEQ yr^{-1}). The top five emitters accounted for 51% of the total releases from all available inventories (86 inventories), demonstrating that population size and economic status have a positive correlation toward PCDD/PCDF releases (see also Pulles et al. [29] and Ren and Zheng [30] for smaller datasets).

Graphical sketches of releases according to economic status are shown in Figs. 3, 4, and 5 for total releases and releases to air ($PCDD/PCDF_{total}$ and $PCDD/PCDF_{air}$). Whereas the normalization to area as mg TEQ per km^2 (Fig. 4) results in a scattered picture, using population-based releases, it can be seen that high-income countries (H) have the lowest PCDD/PCDF releases per capita. Not much difference was found between the other income categories (Fig. 3). Normalization to GNI PPP^{-1} shows that lowest releases are found in higher-income countries (H and UM), whereas the LM and L countries exhibit higher releases (Fig. 5).

Table 8 Summary of total releases and releases to air according to income category (and reference year) (86 inventories)

Income group	# Countries	Sum air (g TEQ yr^{-1})	Sum total (g TEQ yr^{-1})	Pop. *mio.	Land area (km^2)	Average total (mg TEQ $capita^{-1}$)	Average air (mg TEQ $capita^{-1}$)
High income	**22**	**5,238**	**8,281**	**521**	**38,685,052**	**19**	**11**
Asia: BRN, CHN-HKG, SYC, TUR	4	1,256	2,190	76	790,882	26	17
CEE: EST, HRV, LTU, RUS, SVN	5	1,958	2,069	153	17,285,580	21	13
GRULAC: CHL, CUB, VEN	3	833	1,411	55	1,778,030	22	13
WEOG: AUS, AUT, CAN, CHE, DEU, FIN, FRA, NOR, NZL, PRT	10	1,190	2,610	237	18,830,560	15	6.3
Upper middle income	**31**	**11,873**	**25,045**	**1,934**	**29,645,964**	**43**	**23**
Africa: MAR, GAB, MUS, SWZ, TUN, ZAF	6	1,217	3,528	90	2,116,324	57	32
Asia: AZE, CHN, FJI, IRN, JOR, LBN, NIU, PLW, SYR, THA, WSM	11	6,977	13,897	1,470	12,251,099	50	26
CEE: ALB, BGR, BLR, MKD, ROU, SRB	6	762	1,662	52	697,450	43	26
GRULAC: ARG, BRA, COL, ECU, PAN, PER, PRY, URY	8	2,917	5,960	322	14,581,550	23	11

(continued)

Table 8 (continued)

Income group	# Countries	Sum air (g TEQ yr^{-1})	Sum total (g TEQ yr^{-1})	Pop. *mio.	Land area (km^2)	Average total (mg TEQ $capita^{-1}$)	Average air (mg TEQ $capita^{-1}$)
Lower middle income	**28**	**15,357**	**35,661**	**2,026**	**15,256,910**	**50**	**23**
Africa: BEN, BFA, CIV, CMR, CPV, DJI, GHA, GMB, KEN, MLI, NGA, SDN, TGO, TZA, ZMB	15	9,404	16,250	359	8,468,650	60	36
Asia: BGD, IDN, IND, KHM, LAO, LKA, NPL, PHL, TJK, VNM	10	5,932	18,572	1,798	6,741,880	17	6.7
CEE: ARM, MDA	2	19	828	6.7	63,580	115	2.7
GRULAC: NIC	1	191	498	5.4	130,370	93	36
Low income	**5**	**803**	**1,344**	**111**	**2,217,390**	**35**	**22**
Africa: BDI, ETH, LBR, MDG, ZWE	5	803	1,344	111	2,217,390	35	22
Total	86	33,459	70,819	4,736	85,953,345	39	20

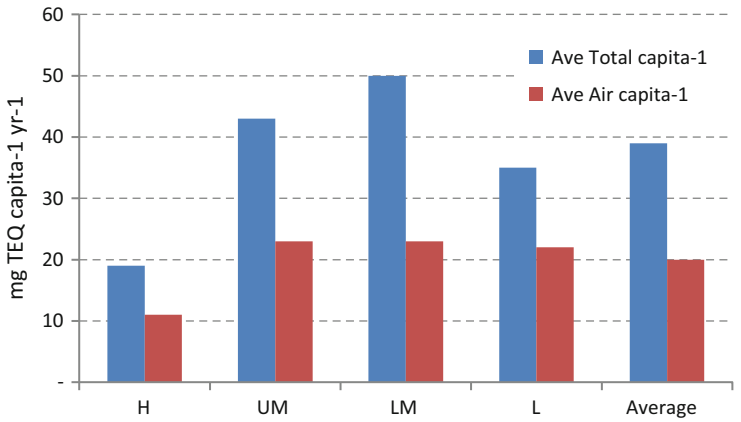


Fig. 3 Average annual releases of PCDD/PCDF per capita and year (mg TEQ $capita^{-1}$ yr^{-1})

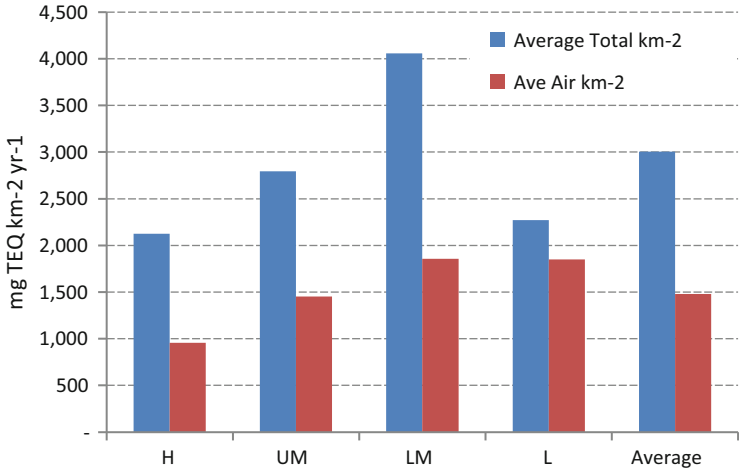


Fig. 4 Average annual releases of PCDD/PCDF per square kilometer and year (mg TEQ km^{-2} yr^{-1})

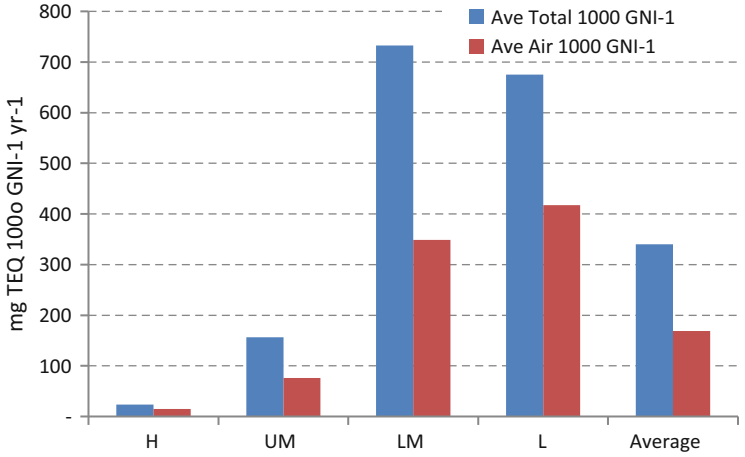


Fig. 5 Average annual releases of PCDD/PCDF per gross national income per capita purchase parity and year (mg TEQ 1,000 USD GNI PPP^{-1} yr^{-1})

4.3 Quantitative Inventory Results Using Source Groups

4.3.1 Releases According to Ten Source Groups

When applying the Toolkit methodology, releases of PCDD/PCDF cannot only be assessed according to the five release vectors but also according to the ten source groups (SG). Of these, nine are quantitative, whereas the tenth source group represents hot spots, which cannot be assigned to a reference year. For the two most important release parameters – total releases and releases to air – the statistics are as shown in Tables 9 and 10 and in Fig. 6, respectively. For both releases, the

Table 9 Descriptive statistics for releases PCDD/PCDF$_{total}$ by source groups

	Total_SG1	Total_SG2	Total_SG3	Total_SG4	Total_SG5	Total_SG6	Total_SG7	Total_SG8	Total_SG9	Total_SG10
Mean (%)	13	11	12	1	2	45	5	1	8	0
Median (%)	6	2	5	0	0	43	0	0	1	0
Std error (%)	2	2	2	0	1	4	1	0	2	0
Std dev. (%)	19	18	18	3	9	33	11	2	15	1
25th percentile (%)	1	0	1	0	0	11	0	0	0	0
75th percentile (%)	17	13	15	1	1	77	4	0	10	0
Minimum (%)	0	0	0	0	0	0	0	0	0	0
Maximum (%)	89	85	80	14	79	100	60	14	97	7
Count	86	86	86	86	86	86	86	86	86	86

Table 10 Descriptive statistics for releases PCDD/PCDF$_{Air}$ by source groups

	Air_SG1	Air_SG2	Air_SG3	Air_SG4	Air_SG5	Air_SG6	Air_SG7	Air_SG8	Air_SG9	Air_SG10
Mean (%)	17	9	15	2	3	51	1	1	0	0
Median (%)	9	3	7	1	1	56	0	0	0	0
Std error (%)	2	2	2	0	1	4	1	0	0	0
Std dev. (%)	23	15	20	4	10	33	5	3	0	0
25th percentile (%)	1	0	2	0	0	24	0	0	0	0
75th percentile (%)	22	10	21	3	2	80	0	1	0	0
Minimum (%)	0	0	0	0	0	0	0	0	0	0
Maximum (%)	100	70	91	20	89	100	45	14	1	0
Count	85	85	85	85	85	85	85	85	85	85

Note: Armenia reported total releases only; therefore, this one country cannot be included here; total of 85 reports

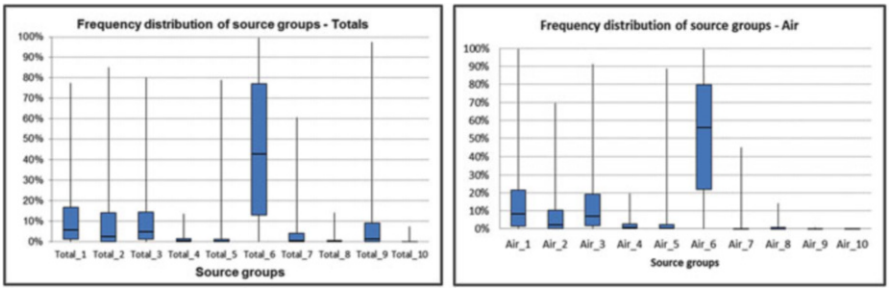


Fig. 6 Frequency distribution of source groups according to country. Source groups are as follows: SG1 = waste incineration, SG2 = metal production, SG3 = power and heat, SG4 = mineral production, SG5 = transport, SG6 = open burning, SG7 = industry, SG8 = miscellaneous, SG9 = disposal, SG10 = hot spots

source group 6, corresponding to open burning processes, is by far the largest source group with a 75th percentile of 77% and 80%, respectively. For total releases, the second and third largest source groups are waste incineration (SG1 = 17%) and metal production and heat and power (SG2 and SG3; both with 14%). For air releases, the second and third largest contributors are waste incineration (SG1 = 22%) and heat and power (SG3 = 19%).

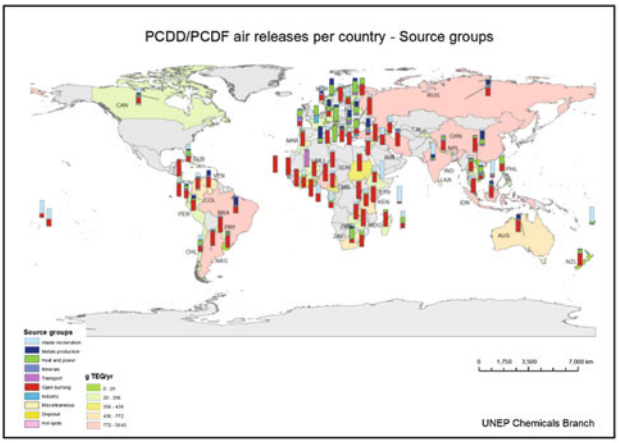
The releases to air, expressed as g TEQ per year according to income categories by source group, are displayed in Table 11. As can be seen, the highest contribution to all air releases was from SG6, open burning processes, such as open burning in agriculture and forests and waste, occurring in lower middle-income countries (>10,300 g TEQ yr^{-1}). Interestingly, SG6 had the highest releases for all income categories. For the high- and upper higher-income groups, the second most important source group is SG2, the production of ferrous and nonferrous metals (relatively biggest emitter SG6). Notably, the industrial process for the production of consumer goods (SG7) is the source group with the lowest releases to air (174 g TEQ yr^{-1}).

Graphical sketches of the distribution of source groups to total and air releases of PCDD/PCDF (g TEQ yr^{-1}) are shown in Fig. 7.

Table 11 Sums of air emissions by source group and income category (g TEQ yr^{-1})

Income group	ΣSG1	ΣSG2	ΣSG3	ΣSG4	ΣSG5	ΣSG6	ΣSG7	ΣSG8	ΣSG9	Subtotals
High	345	1,541	719	553	101	1,833	125	23	-	5,238
Upper middle	1,263	3,417	2,129	708	214	3,983	44	116	0.2	11,873
Lower middle	2,833	1,000	949	243	98	10,366	5	45	1.0	15,545
Low	60	0.2	33	0.4	1.0	708	0	0.1	0.4	803
Total	4,501	5,958	3,829	1,504	413	16,889	174	183	1.6	33,459

Numbers may not add up at last digit due to rounding procedure



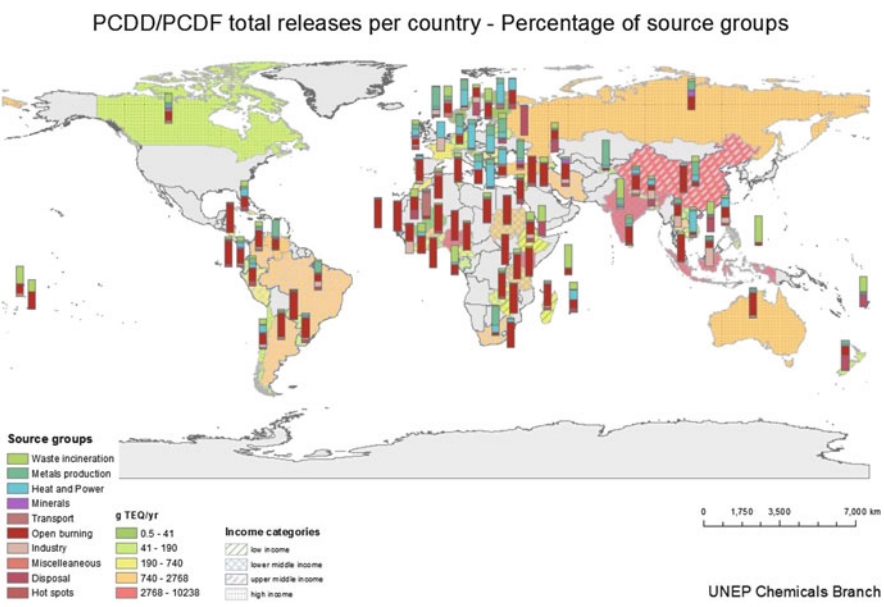


Fig. 7 (continued)

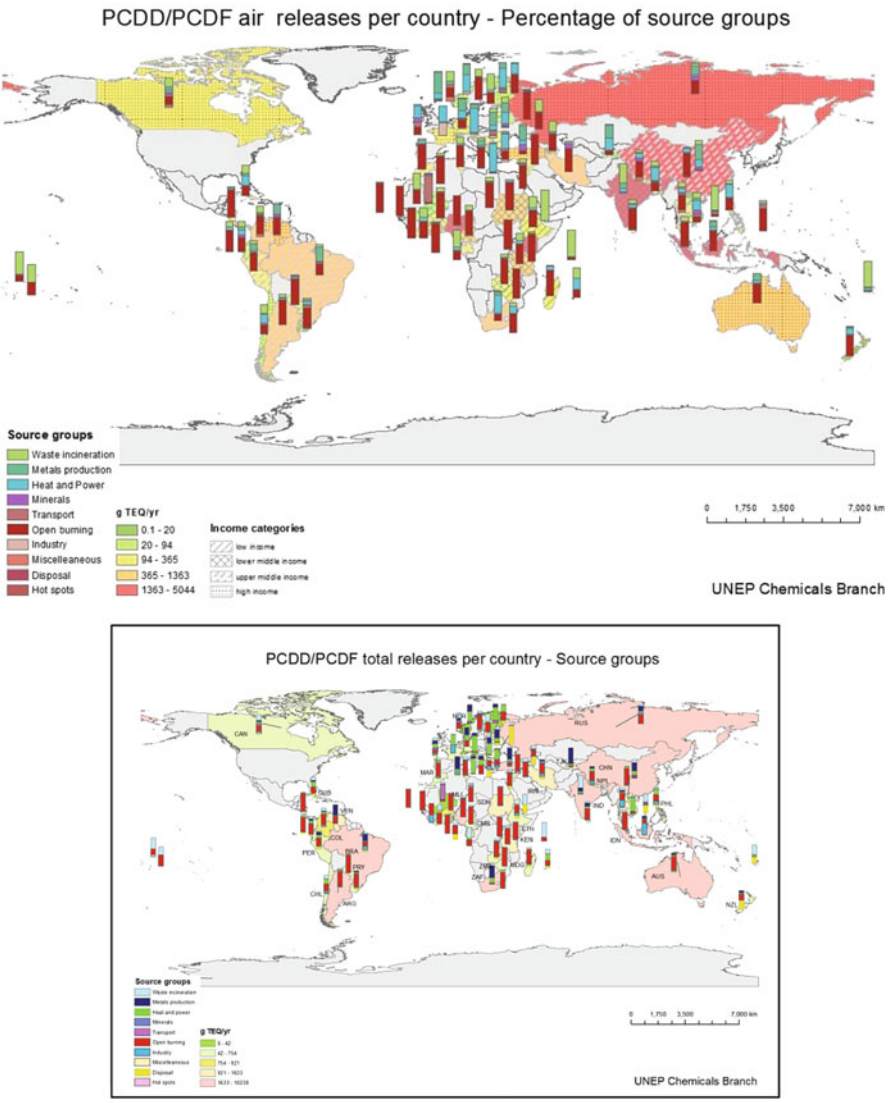


Fig. 7 Presentation of total annual releases and releases to air per source group and income category based on total and air releases, respectively (g TEQ yr^{-1}). For countries colored in *grey*, no information has been provided so far; they are not included in Table 1

5 Conclusions

The development and periodic updating of national release inventories for unintentional POPs is an obligation for countries that have ratified the Stockholm Convention. The Conference of the Parties had endorsed a reporting format according to Article 15 of the Convention where countries report their national inventories for ten source groups and five release vectors. According to schedule in the legally binding instrument, these inventories should be revised and updated every five years. In order to report national inventories in a transparent, complete, and comparable manner, the Toolkit has been developed by UNEP and is being revised and updated periodically. The first round of reporting took longer than anticipated and still does not yet include all parties (86 reported whereas the Convention has 179 parties; status: June 2015). Nevertheless, an abundance of information has been generated from these inventories including political and technical results.

At policy level and for practical reasons, it is recommended that inventory activities be focused on PCDD/PCDF only, as these substances are indicative of the presence of other unintentional POPs (HCB, PCB and PeCBz according to Annex C of the Stockholm Convention). It is also recommended to use the TEQ approach. PCDD/PCDF are considered to constitute a sufficient basis for identifying and prioritizing sources of all these substances as well as for devising applicable control measures for all Annex C POPs and for evaluating their efficacy. Only in the context of research or other projects it is advisable to analyze emissions of all unintentional POPs listed in Annex C in order to produce useful information for the purpose of deriving emission factors [11].

At technical level, across all inventories, the most important release vector is air, receiving 47% of all PCDD/PCDF releases; the second most important vector is residue with 32%. Of the other releases vectors, water does not play a role (receiving only 2% of all PCDD/PCDF releases); releases to land or product account for 11% and 8%, respectively. Among the source groups, SG6 (open burning) constitutes the largest emitter for PCDD/PCDF$_{total}$ (mean = 45%) and for PCDD/PCDF$_{air}$ (mean = 51%), followed by SG1 (waste incineration) with 13% for PCDD/PCDF$_{total}$ and 17% for PCDD/PCDF$_{air}$. The production of minerals (SG4), transport (SG5), industry (SG7, chemical industry, pulp and paper, textile, etc.), and the source group of miscellaneous (SG8, including crematoria, tobacco smoking) do not contribute much to PCDD/PCDF release inventories.

High-income countries tend to have lowest average releases of PCDD/PCDF$_{total}$ and PCDD/PCDF$_{air}$ per capita (19 mg TEQ capita^{-1} yr^{-1} and 11 mg TEQ capita^{-1} yr^{-1}, resp.), whereas the lower middle-income countries have highest releases for PCDD/PCDF$_{total}$ and PCDD/PCDF$_{air}$ (50 mg TEQ capita^{-1} yr^{-1} and 23 mg TEQ capita^{-1} yr^{-1}, resp.; see Table 8). In the high- and upper middle-income countries, metal recycling processes (SG2) are the second highest emitters, numerically very close to the SG6, the largest emitter.

Acknowledgment This review is dedicated to Otto Hutzinger who has been my supervisor at the University of Bayreuth, Germany, for many years and who gave me the chance to undertake "dioxin" research. He introduced me into the network of scientists active in a multidisciplinary area to open my eyes and senses beyond academic approaches and objectives. Together with many friends and colleagues, we value Otto Hutzinger as a researcher, mentor, and great personality.

For this review, I thank colleagues and coworkers, government representatives, students, and interns for assistance in the development of the Toolkit methodology and the wider discussions on pollutants' inventories. Special thanks go to Ms. Haosong Jiao for the preparation of the maps. With the present status, we have come a long way but remain curious and willing to improve further.

Glossary

Box and Whisker plot Graphically summarises the median, a measure of dispersion (first and third quartile) and the range for a sample or population.

GNI PPP Is gross national income (GNI) converted to international dollars using purchasing power parity rates. An international dollar has the same purchasing power over GNI as a U.S. dollar has in the United States. Gross national income is the sum of value added by all resident producers plus any product taxes (less subsidies) not included in the valuation of output plus net receipts of primary income (compensation of employees and property income) from abroad. PPP GNI was named formerly PPP GNP.

Income categories As defined by the World Bank; they are as follows: Low income (L), lower middle income (LM), upper middle income (UM), and high income (H).

ISO codes ISO 3166 is the International Standard for country codes and codes for their subdivisions. The purpose of ISO 3166 is to define internationally recognised codes of letters and/or numbers that we can use when we refer to countries and subdivisions. The ISO country codes are maintained by the ISO 3166 Maintenance Agency, Geneva, Switzerland, www.iso.org/iso/country_codes.

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Environmental Control Challenges of Dioxins, Polychlorinated Biphenyls, and Brominated Flame Retardants

Shin-ichi Sakai

Abstract Over the past few decades, the international community has challenged on persistent organic pollutants (POPs) control issues on both global and local scale. This article deals with problems related to POPs from the standpoint of their total life cycle and examines each category of intentionally and unintentionally produced chemicals. Environmental control challenges and experiences of dioxins, polychlorinated biphenyls, and brominated flame retardants were addressed. Polychlorinated dioxins have their various release sources, but in particular, thermal processes such as waste incineration are the main emission sources. The international community has addressed this issue by adopting technologies for complete combustion and advanced emission gas treatment. With these technologies, Japan has successfully achieved more than 95% reduction of dioxin emission. PCB-containing products manufactured in the past are thought to be the main source of PCB presence now in the environment. Thermal destruction and chemical dechlorination processes have been applied for such waste PCBs. It becomes clear that brominated flame retardants (BFRs) accumulate in the environment as well as in humans. Global-scale efforts have just begun to control BFRs. From now on, we should also address the problem of brominated dioxins and try to improve the related technologies and/or develop new technologies also in the future.

Keywords Polychlorinated dibenzodioxins and dibenzofurans (PCDDs/DFs), Polychlorinated biphenyls (PCBs), Brominated flame retardants, Polybrominated dibenzodioxin and dibenzofurans (PBDDs/DFs), Environmental control, Product life cycles

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Contents

1 Introduction

Toxic substances and persistent organic pollutants (POPs) have the potential to cause negative effects on human health and the environment. In the late twentieth century, there were many environmental pollution problems caused by toxic substances and POPs, including the Love Canal incident in the United States, the Seveso disaster in Italy in the 1970s, and the Teshima incident in Japan in the 1980s. Such incidents, which raised concerns about negative impacts on human health and the environment, had a major impact on people's peace of mind and led to detection of POPs on a global scale. At the World Summit on Sustainable Development (WSSD) in 2002, there was a renewed commitment, initially advanced in Agenda 21, to the sound management of hazardous wastes and of chemicals throughout their life cycle, the goal being sustainable development and the protection of human health and the environment [1]. As stated at the WSSD, one of the crucial issues for the world is sound management of chemicals and hazardous wastes.

Over the past few decades, the international community has made efforts and taken action to control POPs, because they are one of the causes of environmental problems on a global scale. This article deals with problems related to POPs from the standpoint of their life cycle and examines each category of intentionally and unintentionally produced chemicals. Each control measure that targets emissions of dioxins and PCBs will be considered, and the results of substance flow analyses for PCBs will also be introduced. In recent years, efforts have been initiated to prevent the use of some brominated flame retardants. The idea of controlling POPs through clean, cycle, and control concepts will also be discussed.

2 Persistent Organic Pollutants and Product Life Cycles

POPs have been detected in the environment throughout the world; they have been passively transported and have accumulated in the environment in places as remote as the North and South Poles. To address this global concern, the Stockholm Convention on Persistent Organic Pollutants was adopted in May 2001 [2]. Most

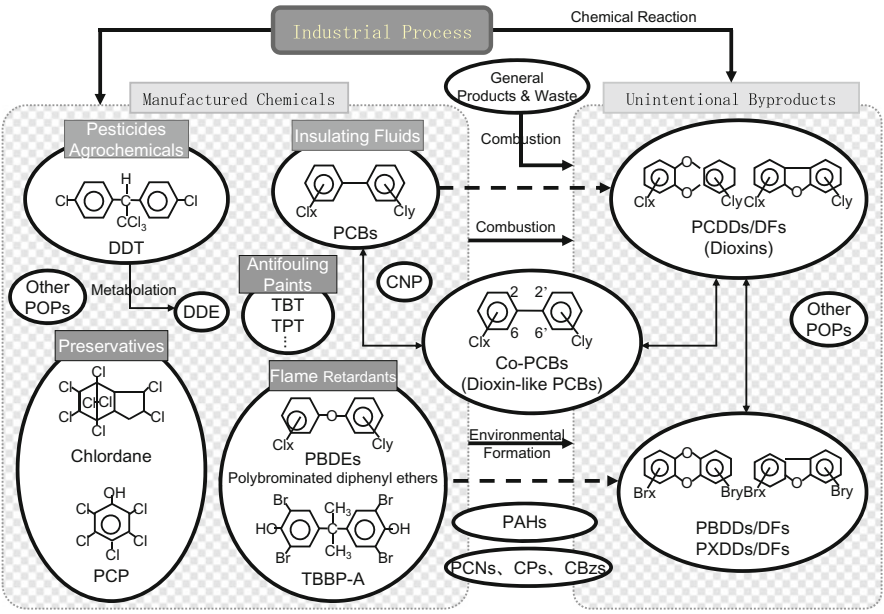

Fig. 1 Two categories of persistent organic pollutants: manufactured chemicals and unintentional by-products

detected POPs have been derived from human activities; they are less likely to be created in nature. As shown in Fig. 1, POPs produced by various activities can be categorized into two groups:

1. Chemicals intentionally produced for industrial and commercial uses
2. Chemicals unintentionally produced as by-products during chemical reactions and combustion

Representative substances classified in category 1 are polychlorinated biphenyls (PCBs) used as insulating fluids (e.g., transformer oil) and heat-resistant media and hexachlorobenzene (HCB), which is used as an intermediate in the production of solvents. In agriculture, aldrin, dieldrin, endrin, and DDT have been used as pesticides. DDT has even been used to control diseases such as malaria. As a result, there have been cases where these substances have been released into the environment through direct spraying of pesticides on agricultural land, accidental leakage of chemicals, and dumping of waste products. Among the major chemical substances classified in category 2 are dioxins (polychlorinated dioxins and dibenzofurans). Dioxins are formed as by-products and are released into the environment during the chemical manufacturing of pesticides or combustion of waste and refining of metal. Figure 1 includes substances other than the 12 POPs (aldrin, dieldrin, endrin, chlordane, heptachlor, toxaphene, mirex, hexachlorobenzene, PCBs, DDT, dioxins, and furans) specified under the Stockholm Convention.

It is essential to examine which types of POPs cause problems during product life cycles. First, there are chemicals that are intentionally produced; these chemicals include PCBs and HCB for industrial use and DDT and other substances used for disease control. Substances used in closed systems and chemicals that can be recovered and for which the purpose of use is clear should be collected and destroyed from now on. In contrast, it is hard to identify proper treatment for substances that have been used in open systems. However, it should at least be required that their effects on the environment be examined. Second, there are unintentionally produced chemicals such as dioxins and HCB that are generated during manufacturing processes. The targeted objects are dioxins created as the by-products of chemical reactions involved in the manufacturing of pesticides and from combustion reactions during metal refining. HCB is sometimes present in residual manufacturing solvents or as an impurity in pesticides. Third, there are by-product problems associated with waste management. Dioxins produced in combustion processes have been acknowledged to be especially serious problems. It is also known that PCBs and chlorinated benzenes are produced unintentionally during combustion. Measures need to be taken to control the production of all of these toxic by-products. Fourth, degradation of the waste generated by the above processes should be required. Waste pesticides and intentionally produced, recycled POPs such as waste PCBs and chlordane should, in particular, be destructed by chemical and/or thermal technologies. In addition, what is most important for establishing sound policies for material cycles is that diffusion of POPs be restricted as much as possible when materials containing them are recycled. Especially careful attention should be paid when using feedstuffs, agricultural land, and recycled resources such as indoor materials. Care should be taken to avoid the possibility of exposing children to toxic chemicals and to prevent toxic chemicals from entering groundwater.

3 Chlorinated Dioxin Control

Chlorinated dioxins generally indicate polychlorinated dibenzo-*p*-dioxins (PCDDs), of which there are 75 congeners distinguished by the number of chlorines and their substituent positions. Some of the congeners have chlorines at the 2, 3, 7, or 8 positions and are very toxic. Polychlorinated dibenzofurans (PCDFs), which have characteristics similar to PCDDs, include 135 congeners. This report refers to both PCDDs and PCDFs as dioxins. PCDDs/DFs are produced as by-products in the manufacture of chemicals such as the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and the antiseptic pentachlorophenol (PCP), and traces of PCDDs/DFs are present in these chemicals. The herbicide 2,4,5-T was used extensively as a defoliant in the Vietnam War, and its use caused great concern because of the suspected relationship between its use and the appearance of deformed children [3]. Even before the latter half of the 1970s, accidents at chemical plants and inadequate treatment of chemical waste have exposed people to PCDDs/DFs.

Examples include the Seveso accident in Italy and the Love Canal and Times Beach incidents in the United States.

The relationship between PCDDs/DFs and municipal solid waste (MSW) incineration, however, was not pointed out until 1977, when Olie and Hutzinger reported that PCDDs/DFs had been detected in the fly ash of MSW incinerators [4]. It is now widely recognized that the sources of PCDDs/DFs are diverse and include industrial processes as well as MSW incinerators. The known sources of PCDDs/DFs include the following: industrial activities, e.g., chemical processes involving chlorine and bleaching of paper pulp; combustion activities, waste incineration; metal smelting; home heating; and fires. Secondary sources include sludge, compost, and contaminated soil. A global inventory of sources of PCDDs/DFs has shown that MSW incinerators are a major source in every country. European countries, which took measures to address the PCDDs/DFs problem earlier than other regions, have focused on the biggest source of PCDDs/DFs, MSW incinerators, as a part of countermeasures to address dioxin pollution problems. In Germany and the Netherlands, treatment of combustion and waste gases had been expected to reduce the load to 1% of the load at the beginning of the 1990s [5]. In fact, Germany reported a decrease from 400 g of toxic equivalent (TEQ) per year to 50 g TEQ/year from 1993 to 1995 and in 1997 achieved 4 g TEQ/year, a 99% reduction [6]. The Netherlands has made similar improvements. They succeeded in reducing their emissions of PCDDs/DFs from MSW incineration by 99.3% from 1990 to 1995 [7]. As a result, in the Netherlands, the total emissions of PCDDs/DFs from MSW incineration were 4–7% of the total emission in 1995 compared to 79% in 1990. During this time, there was a remarkable increase in European countries of the share of industrial processing, including the sintering of iron ore and iron products and the processing of nonferrous metal, but much progress was made in reducing emissions of PCDDs/DFs from MSW incinerators.

Looking back on the development of world policy concerning PCDDs/DFs over the past 30 years and on environmental dioxin policy, I would like to clarify the implications and issues we face. First, technological standards have been raised. It should be pointed out that the establishment of technological standards is becoming common throughout the world. This trend has been a countermeasure against emissions of PCDDs/DFs. In 1986, Sweden set an emission gas standard of 0.1 ng TEQ per normal cubic meter (Nm^3). Then the Netherlands, Germany, and Austria adopted the same standard and have advanced the emission control measures. Between 1995 and 1997, the United States and Japan adopted almost the same standards.

A key point in controlling technological measures is to apply state-of-the-art technologies to waste gas treatment. Some examples are the use of fabric filters for trapping particles, activated carbon for the adsorption of gases, catalytic decomposition technology for the destruction of gases and conversion of nitrogen oxides to N_2, and waste gas scrubbers, which are also effective in removing SO_2. Complete combustion technology should also be used. Some effective technologies that have been developed to the level of practical feasibility are listed among the high-tech treatment technologies in Fig. 2. They are associated with three principal

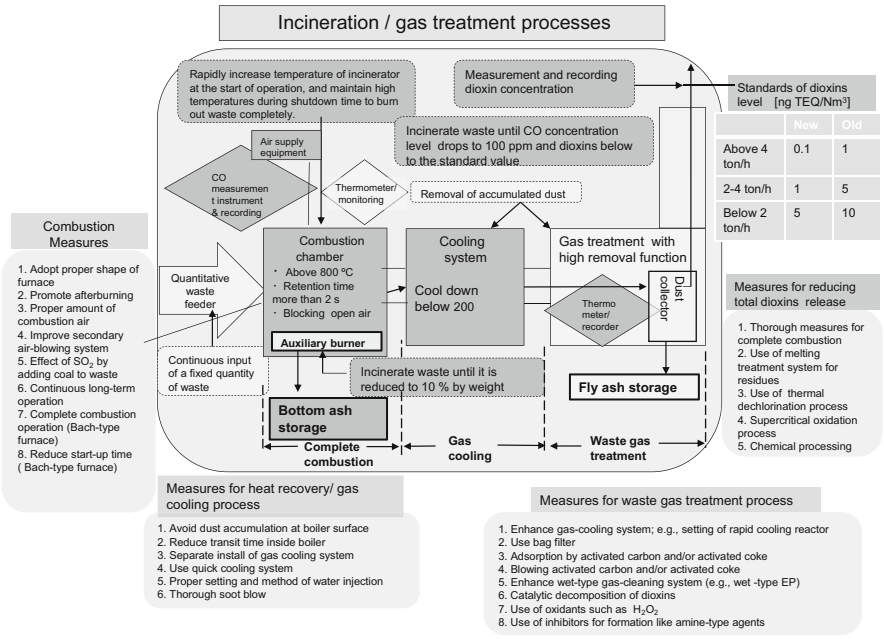

Fig. 2 Dioxin control technologies and standards for incineration/gas treatment

categories: de novo synthesis control technologies, additional technologies for waste gas treatment, and residue destruction and recycling technologies. These advanced technologies have been adopted to promote the application of concepts such as BDAT (Best Demonstrated Available Technology) and MACT (Maximum Achievable Control Technology).

Figure 3 shows the amount of dioxins emitted from various sources in Japan from 1997 to 2009 and the 2010 target rate [8]. The total amount of waste-derived dioxins emitted into the air or water from municipal waste treatment facilities, industrial waste treatment facilities, and small waste incinerators with a treatment capacity of less than 200 kg/h are included in the total. In 1997, when new guidelines were established, the amount of waste-derived dioxins emitted was 7.7 kg of World Health Organization (WHO) TEQ/year and accounted for 94% of the total dioxin emissions from all sources. In 1998, when urgent measures were implemented, the amount was reduced to 3.8 kg of WHO-TEQ/year, a reduction of more than 50%. In 2004, after the promulgation of permanent criteria, the amount was further reduced to 230 g of WHO-TEQ/year, only about 3% of the 1997 emissions. The ratio of incinerator-generated dioxins to the total amount of dioxin emissions from all sources also decreased to 64%. Although it is necessary to pay attention to transport of pollutants through the environment at both the local and global levels, we should first focus on the atmosphere as a conduit, because these pollutants are very mobile after entering the air.

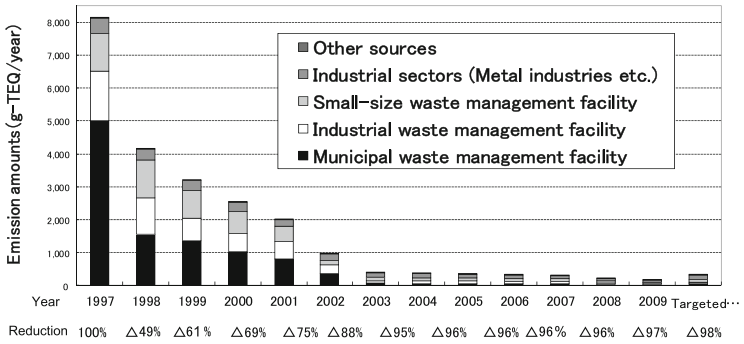

Fig. 3 Amounts of dioxins discharged in Japan from 1997 to 2009 [8]

Table 1 shows atmospheric dioxin concentrations from 1997 to 2004 [9]. The average concentration was 0.55 pg-WHO-TEQ/Nm^3 in 1997; this decreased to 0.23 pg-WHO-TEQ/Nm^3 in 1998. It then gradually decreased to 0.059 pg-WHO-TEQ/Nm^3 in 2004, or about 10% of the 1997 level. No significant differences were found in atmospheric levels of dioxins in the general environment, in the vicinity of emission sources, and along roadsides. The effect of the urgent measures implemented in 1998 is apparent in the reduction of the dioxin concentrations in the air.

The PCDDs/DFs emitted by the various sources, including those emitted to the atmosphere from MSW incineration, are first adsorbed onto the surface of particles and food and from there find their way into soil, water, and biomedia. Once PCDDs/DFs enter environmental media from phases other than air, they enter the air because of their characteristic volatility. Bioconcentration then occurs, because PCDDs/DFs are concentrated in fat and are resistant to biodegradation. They are present in food, including meat and fish.

Source control to reduce the environmental concentrations of PCDDs/DFs should be considered as part of environmental policy, along with recycling and environmental cycle control. PCDDs/DFs are unintentional by-products, and PCDDs/DFs are of no benefit to humans or the environment, and no emissions and no uptake of PCDDs/DFs would be desirable. The historical trends of PCDDs/DFs reveal that human beings have been exposed to them for more than 100 years. The problem is that human activities have raised background concentrations by a factor of 10 in industrial societies during the past century. Figure 4 shows future strategies for controlling PCDDs/DFs emissions based on fundamental scientific understanding. First, control of the source of emission is important. Control measures are required not only for MSW incineration, regulation of which has become very strict, but also for other sources. With respect to waste management, cooperation must be promoted with extant recycling efforts. The strategy is to focus on waste reduction and recycling. This strategy requires consideration of the following two issues: steps must be taken to effectively control PCDDs/DFs emissions during the incineration process, and the steps taken must not shift PCDDs/DFs problems

Table 1 Trends of dioxin concentrations in the ambient environment (pgTEQ/m^3) [9]

		1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
General environment	Average	0.55	0.23	0.18	0.14	0.14	0.093	0.064	0.058	0.051	0.051	0.041	0.035	0.031	0.031	0.028
Surroundings of release sources	Average	0.58	0.20	0.18	0.15	0.13	0.092	0.078	0.063	0.055	0.050	0.040	0.041	0.035	0.036	0.032
Roadside	Average	0.47	0.19	0.23	0.17	0.16	0.091	0.076	0.055	0.054	0.050	0.044	0.036	0.031	0.028	0.025

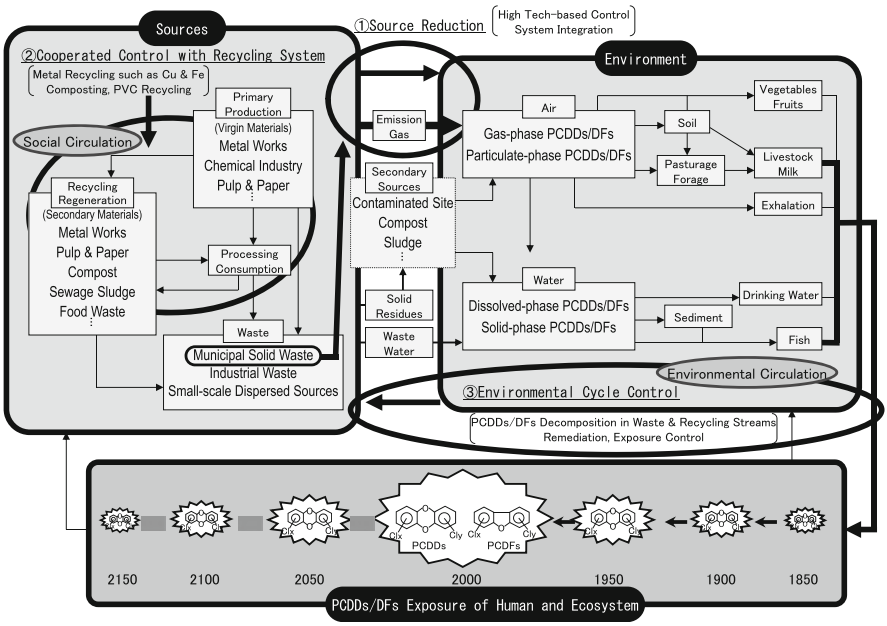

Fig. 4 PCDDs/DFs emission, environmental transport and exposure, and some control points

associated with MSW incineration to problems associated with the recycling process. The recycling of metals, such as copper and iron, leads to a reduction of catalysts that facilitate PCDDs/DFs formation during MSW incineration, and PVC recycling contributes to control of dioxin formation. However, the production of PCDDs/DFs during the process of metal smelting is a problem associated with metal recycling. If recycling is promoted as a measure against the generation of waste, control of recycling should be implemented simultaneously. Therefore, cooperative control with the recycling process is important. Humans and most other living organisms have already been exposed to PCDDs/DFs to a certain extent in the environment. PCDDs/DFs circulate in various environmental media, enter human bodies, and undergo multiple bioconcentrations. This means that long-term reduction of PCDDs/DFs in environmental cycles is important. The following two methods are considered to be the principal strategies for controlling the environmental cycle of PCDDs/DFs. The first method is environmental remediation of hot spots, such as contaminated soil, to prevent local high-level exposure of humans and the ecosystem. The second method is to reduce the amount of environmental cycling by decomposing POPs at appropriate points in their cycle. If waste incineration is the biggest source of PCDDs/DFs, it is critical to change the waste incineration system so that the output of PCDDs/DFs is less than the input. The same strategy applies to many recycling processes. If materials contaminated with PCDDs/DFs can be decomposed through recycling or by incineration, the system deserves to be called environmental cycle control. It would be effective in

reducing not only PCDDs/DFs but also various other hazardous chemical substances, including other dioxin-related compounds and polycyclic aromatic hydrocarbons. Following an environmental strategy based on scientific understanding should be one of the effective countermeasures against PCDDs/DFs among the three long-term strategies, the other two being development of control measures and cooperative controls between recycling and control of environmental cycles. These three strategies must be implemented. As shown at the bottom of Fig. 4, the PCDDs/DFs burden in human beings and other living organisms must be reduced in the next generation through these control measures.

4 PCB Destruction and Environmental Control

PCBs are chemical substances that possess excellent insulation properties. They are poor conductors of electricity and are flame resistant. They have been widely used in electrical equipment such as transformers and capacitors. In 1966, PCBs were first detected in fish and seabirds in many parts of the world [10, 11]. Subsequently, it became obvious that PCB transport was expanding across the world. The toxicity of PCBs first became widely known as a result of the Yusho incident in 1968. In that industrial accident in Japan, PCBs used as a heating medium leaked into rice bran oil during the manufacturing process, and people who consumed the contaminated oil suffered significant health problems [12]. As a result, PCB manufacture was discontinued, and the government required that waste PCBs be collected. In 1973, the Law Concerning the Examination and Regulation of Manufacture of Chemical Substances (Chemical Substances Control Law) was established in Japan, and a framework was created to review and regulate the use of chemical substances. The Chemical Substances Control Law bans the use and manufacture of PCBs and requires the safe storage of PCBs. It has been estimated that about 54 kilotonnes (kt) of PCBs were used in Japan from 1954 to 1972, the period during which they were most commonly used.

The toxic nature of PCBs is a threat to human health and the environment at the present time. PCBs also pose a threat of global environmental pollution in future years because they are widely disseminated through the air and mobile living species. Internationally, the Stockholm Convention on Persistent Organic Pollutants was signed in May 2001. This Convention stipulates that governments have to phase out the use of PCBs by 2025 and dispose of PCBs in an environmentally sound manner no later than 2028. In Japan, the Law Concerning Special Measures against PCB Waste was enacted in 2001 to promote secure and appropriate treatment of PCB waste.

Waste PCBs can be destroyed by noncombustion technology (e.g., chemical treatment) instead of incineration or high-temperature pyrolysis. The criterion for treating PCB-containing oil by chemical decomposition was set at a strict limit of 0.5 mg/kg. PCB treatment has been working very well on the basis of this national program. The Japan Environmental Safety Corporation (JESCO) is a special

Table 2 PCB chemical treatment methods [14]

Facility	Pretreatment	PCB decomposition
Kitakyushu (Phase 1)	Precise recovery cleansing method	Dechlorination method
	Vacuum thermal recycling method (VTR method)	Sodium dispersion method (SD method)
(Phase 2)		Plasma melting method
Toyota	Solvent extraction decomposition method (SED method) (includes vacuum heating separation method)	Dechlorination method
		Ontario hydro technologies sodium dispersion method (OSD method)
Tokyo	Chemical cleansing method (includes vacuum heating separation method)	Hydrothermal oxidation decomposition method
		Hydrothermal decomposition method
Osaka	Solvent cleansing method	Dechlorination method
	Vacuum thermal recycling method (VTR method)	Catalyst hydrogenation dechlorination method (Pd/C method)
Hokkaido	Solvent extraction decomposition method (SED method) (includes vacuum heating separation method)	Dechlorination method
		Sodium dispersion method (SP hybrid method)

company wholly owned by the central government. It was established in April 2004 under the Japan Environmental Safety Corporation Law (Law No. 44 of 2003) to conduct treatment of PCB waste as its principal business activity [13]. At the present time, JESCO treats PCB wastes at five PCB facilities. The first was the Kitakyushu facility, which started operations in 2004. It was followed by the Toyota, Tokyo, Osaka, and Hokkaido facilities. In accordance with the company policy to carry out PCB waste treatment with a priority on safe and reliable treatment and full information disclosure, JESCO has designed its facilities in accord with the use of safe and sure treatment methods, use of multiple safety measures based on risk management concepts, and disclosure of information about treatment status. An outline of treatment methods used at JESCO facilities is shown in Table 2. All JESCO facilities use only chemical decomposition methods, because they do not produce combustion gases. The Tokyo facility has adopted the "hydrothermal oxidation decomposition method," and the other four facilities have adopted the "dechlorination method."

In order to ensure that PCB destruction is being accomplished satisfactorily, it is important not only to ascertain the disappearance of PCBs but also to know the mechanism associated with the degradation and to check for the absence of other harmful by-products [15]. Thirteen PCB isomers [2-chlorobiphenyl (#1), 3-chlorobiphenyl (#2), 4-chlorobiphenyl (#3), 2,3,4-trichlorobiphenyl (#21), 2,4,4′-trichlorobiphenyl (#28), 2,2′,5,5′-tetrachlorobiphenyl (#52), 2,2′,4,5,5′-pentachlorobiphenyl (#101), 2,3′,4,4′,5-pentachlorobiphenyl (#118), 3,3′,4,4′,5-pentachlorobiphenyl (#126), 2,2′,3,4,4′,5′-hexachlorobiphenyl (#138), 2,2′,4,4′,5,5′-hexachlorobiphenyl (#153), 2,2′,3,4,4′,5,5′-heptachlorobiphenyl

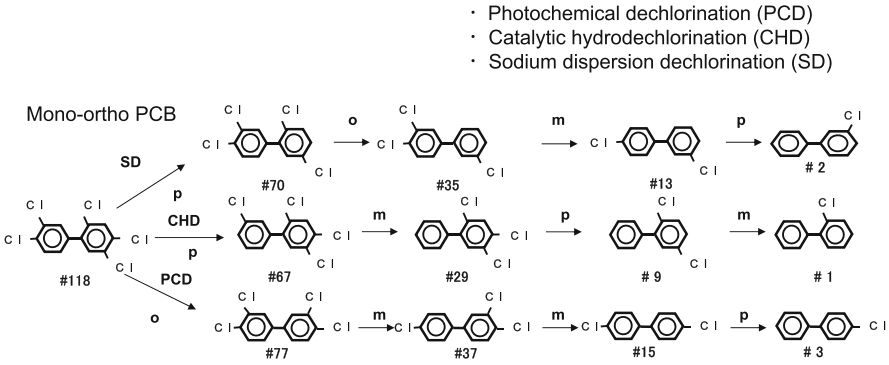

Fig. 5 Dechlorination pathways of 2,3′,4,4′,5-pentachlorobiphenyl (#118) [15]

(#180), and decachlorobiphenyl (#209)] were decomposed by three noncombustion methods, catalytic hydrodechlorination (CHD) over a palladium/carbon catalyst, photochemical dechlorination (PCD), and a sodium dispersion (SD) method. The reaction solutions were sampled at intervals during the decompositions and analyzed. On the basis of the identification of the dechlorinated products and the quantitative data obtained during the reactions, the major dechlorination pathways were proposed. Consideration was given to the differences in the pathways and the reactivities of the chlorines between the three methods.

The differences of the degradation mechanisms among the three methods have been compared. The dechlorination pathways of 2,3′,4,4′,5-pentachlorobiphenyl (#118) are shown in Fig. 5. Although the dechlorinations proceeded via an irreversible stepwise pattern in all three cases, the dechlorination pathways were quite different among the three methods. Whereas the dechlorination pathways were relatively clear via CHD and PCD, the pathways diverged widely in the case of SD. The chlorine at the ortho position was easily dechlorinated via PCD and was removed most slowly via CHD. The chlorine in the para position was removed slightly more easily via SD. The congeners with more chlorines decomposed most quickly and in proportion to the total number of chlorines via SD. This effect was not apparent with the CHD and PCD methods because the presence of ortho chlorine atoms had a large effect on the degradation constants. Furthermore, the results were checked to determine whether dioxin-like PCBs were destroyed and/or produced. When 2,3′,4,4′,5-PeCB (#118) as the mono-ortho congener was destroyed via CHD and SD, the total TEQ also decreased immediately. In the case of the destruction of #118 via PCD, the TEQ caused by 3,3′,4,4′-TeCB (#77) increased in the beginning of the experiment because #77 was the principal product of orthodechlorination of #126. The TEQ, however, gradually decreased with the degradation of #77 and finally became zero. The total TEQ decreased immediately via CHD and SD, and after it gradually decreased via PCD, it finally became zero by all three methods.

Dioxin-like PCBs in the environment can be divided into dioxin-like PCBs derived from products containing PCBs (hereafter, PCB products) and unintentionally generated dioxin-like PCBs. PCB products derived from dioxin-like PCBs enter the environment from equipment that contains PCBs, as well as from improper storage and illegal dumping of PCB wastes. The unintentional generation of dioxin-like PCBs occurs mainly in association with chemical reactions and waste incineration [16, 17]. Comparison of the two types reveals significant differences in congeners and their relative abundance. Dioxin-like PCBs penetrated widely into the environment during the period when PCB products were produced and used, and they also accumulated, primarily in marine areas and lakes.

Well-known sources of dioxin-like PCBs include those released by the use or disposal of industrial PCB products or formed as by-products during MSW incineration. It is also well known that PCBs are thermally decomposable. In Japan, 5.3 kt of waste liquid PCBs, namely, Kanechlors, were thermally destroyed at the Takasago plant of Kaneka Co. Ltd. in 1988. In European countries and the United States, waste PCBs are regularly incinerated at high temperatures. MSW incineration processes have the potential to both produce and destroy PCBs. The results of substance flow analyses have previously been reported from a MSW incineration facility in Kyoto City [16]. The existing MSW incinerator was regarded as a system, and the amounts of dioxin-like PCBs and other PCBs in the MSW inflow and the amounts released via gas emissions and incineration residues were examined. A substance flow analysis for dioxin-like PCBs in a newly constructed facility was also performed, and the results were compared with those from an existing facility. To take into account sources of dioxin-like PCBs in the atmosphere, the bulk deposition of dioxin-like PCBs was measured and compared to amounts released by MSW incineration in the Kyoto City area [17].

To investigate whether dioxin-like PCBs and total PCB homologue groups tended to be formed or decomposed in the MSW incineration facilities, the release/inflow ratio (defined as the value of the amount released in emitted gases, fly ash, and bottom ash divided by the amount of the inflow) was calculated for each congener and homologue, as shown in Fig. 6. For the newly constructed facility, the release/inflow ratios of PCB congeners 126, 169, and 189 were still greater than 1, the implication being that levels of these compounds increased during incineration. For the other congeners, the ratios were less than 1, indicating that these compounds were destroyed within the system. The ratios expressed in terms of TEQ levels were greater than 1. This was mainly due to the greater contribution of congener 126 to the TEQ. However, the total amounts of dioxin-like PCBs and PCB homologues showed a decreasing tendency.

To investigate the behavior of dioxin-like PCBs in the atmosphere, the amount released into the air through MSW incineration was compared with the amount deposited from the air to the ground. The results for dioxin-like PCBs (Fig. 7) were very different from other PCB congeners and homologues. For congeners 81, 126, 169, and 189, the ranges of the amounts deposited fell within the ranges of the amounts released through waste incineration. Conversely, for congeners 105, 114, and 118, the ranges of the amounts deposited were much higher than the ranges of

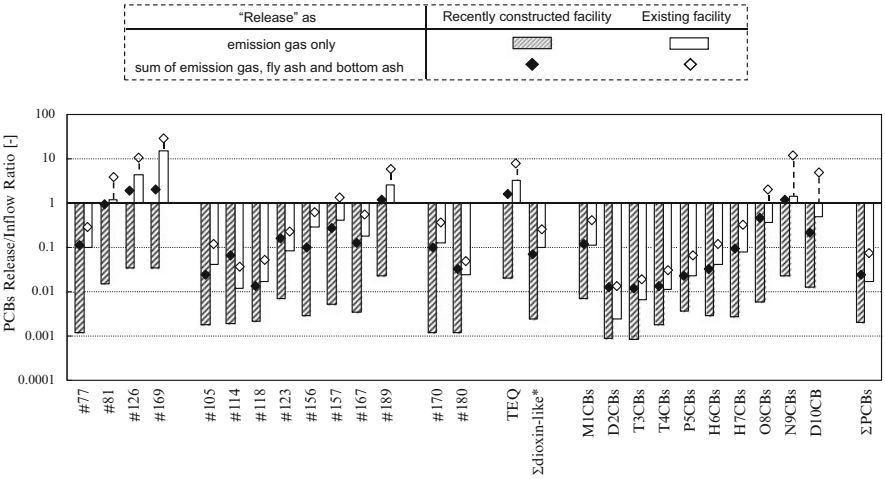

Fig. 6 PCB release/inflow ratio of MSW incineration [17]

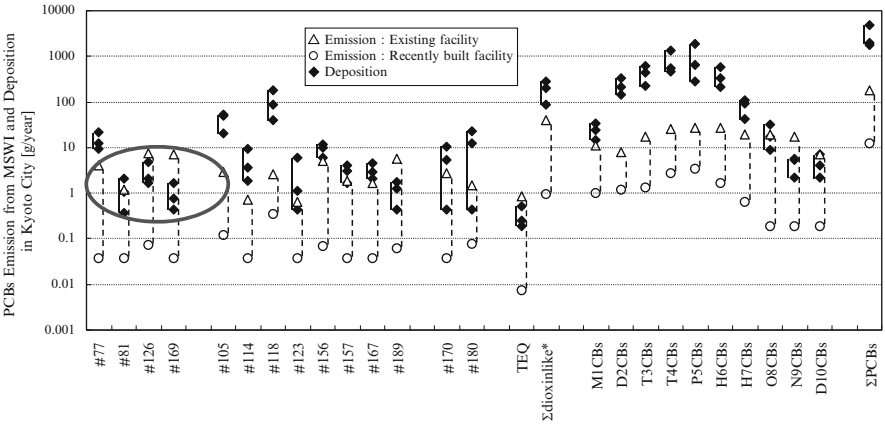

Fig. 7 Comparison of dioxin-like PCBs in incinerator emissions and in depositions in Kyoto City [17]

the amounts released through waste incineration. Summarizing the patterns of each congener and homologue together, the results could be roughly classified into three groups:

Group 1: Congeners and homologues for which the amounts deposited were much higher than the amounts released through waste incineration, dioxin-like PCBs 105, 114, and 118 and PCBs D_2CBs to H_7CBs

Group 2: Congeners and homologues for which the amounts deposited were of the same order of magnitude as the amounts released through waste incineration,

dioxin-like PCBs 81, 126, 169, and 189, PCBs O_8CBs to D_{10}CB, PCDDs/DFs 2,3,7,8-substituted congeners, and all homologues except TeCDDs and PeCDDs
Group 3: Those falling between the two previous groups

With reference to group 1, congeners 105 and 118 are found in high concentrations in the industrial PCB product Kanechlor. Dioxin-like PCBs 170 and 180 are very abundant in the highly chlorinated PCB product KC-600. With regard to the congener profiles of dioxin-like PCBs in the atmosphere, percentages of congeners 77, 105, 118, and 180 are high, and their trends are similar to those of industrial PCB products. As for PCB homologues, the amounts of T3CBs to H7CBs are very high in industrial PCB products. With reference to group 2, the percentages of nonortho dioxin-like PCB congeners are high in incinerator emission gases. As mentioned before, the release/inflow ratios of these congeners were greater than 1, the suggestion being that these congeners tend to form during the waste incineration process. The release/inflow ratios of O_8CBs to D_{10}CB were higher than the ratios of D_2CBs to H_7CBs. Conversely, congeners 126, 169, and 189 and O_8CBs to D_{10}CB are very rarely found in industrial PCB products. From the above findings, the general trend is assumed to be as follows: (1) For congeners found in high concentrations in industrial PCB products, the amounts deposited are much higher than the amounts released through incinerator emission gases (group 1). (2) For congeners commonly found in waste incineration emission gases, the amounts deposited are similar to the amounts released with the emission gases (group 2).

When PCB regulation began, the concentrations of dioxin-like PCBs in the environment decreased. Because of this reduction, the proportion of incineration-derived, dioxin-like PCBs has increased. The use of advanced dioxin control measures has drastically reduced the amount of incineration-derived, dioxin-like PCB formation, but the level of dioxin-like PCBs remains high in fish and seafood. Therefore, control of dioxin-like PCBs is considered to be crucial for preventing human exposure to PCBs.

5 BFRs and PBDDs/DFs Control

Flame retardants are used to protect the public from accidental fires by reducing the flammability of combustible materials such as plastics, synthetic polymers, and textiles [18]. The most important group of flame retardants is the brominated flame retardants (BFRs), which represents various chemicals. Some BFRs, such as polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBP-A), and hexabromocyclododecane (HBCD), have been studied with respect to environmental pollution and human exposure for the past three decades. Recently, environmental problems related to BFRs have become a matter of greater concern than ever before. One of the reasons is the marked increase in PBDE levels observed in human milk in Sweden [19] and North America [20]. In particular, the PBDE levels in human milk around the year 2000

in North America were reported to be two orders of magnitude higher than those in Sweden or Japan. Another reason for concern is the recent analysis of toxicological data that has demonstrated that some BFRs have the potential to cause health effects because they have thyroidogenic, estrogenic, and dioxin-like activities [21]. The emerging problem is therefore whether environmental levels of PBDEs and other BFRs will continue to increase and possibly cause toxic effects in humans.

For the past several decades, another social concern related to BFRs has been their breakdown products, which include polybrominated and mixed brominated/chlorinated dibenzo-*p*-dioxins and dibenzofurans (PBDDs/DFs and mixed PXDDs/DFs). The German Government amended its existing Hazardous Substance Ordinance in 1994 to include eight 2,3,7,8-substituted PBDDs/DFs. The WHO has published a document concerning brominated dibenzo-*p*-dioxins and dibenzofurans [22]. This document includes and reviews studies on the physical and chemical properties, methods of formation and sources, environmental behavior, environmental levels and human exposure, kinetics and metabolism, and toxicity of PBDDs/DFs and mixed PXDDs/DFs. The WHO concluded that PBDDs/DFs are contaminants that are more or less similar to polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/DFs) in terms of their persistence and toxicity and that humans and the environment should be protected from these compounds.

Control of BFRs and PBDDs/DFs is the target of the fields of recycling and waste management of e-waste and end-of-life vehicles (ELVs). Automobile ownership worldwide exceeded one billion in 2010. The generation of ELVs was estimated at 40 million, which accounts for 4% of total automobile ownership [23]. ELVs are recycled as secondary materials and disposed of as waste. The recycling rate of ELVs in general ranges from 75% to 80%. In normal recycling and disposal procedures, ELV bodies are crushed, and the automobile shredder residue (ASR) remaining after the recovery of metals is disposed of mainly in landfills. However, landfills for industrial waste are becoming increasingly scarce. ASR has the following characteristics: (1) it has a high calorific value and high ash content; (2) it contains many fine particles with diameters of 5 mm or smaller as well as considerable crushed waste with diameters of 50 mm or larger, the result being a low bulk specific gravity; and (3) it contains large amounts of heavy metals, BFRs, and chlorine. Because of these characteristics, ASR is considered to be a type of waste that is very difficult to treat. ASR treatment requires an evaluation of the technology needed to decompose and control emissions of toxic substances. Osada et al. conducted an ASR melting test using a shaft-type direct melting furnace to identify the behavior of the BFRs and PBDD/DFs and the distribution of heavy metals in the slag and fly ash [24].

The test facility consisted of a melting furnace, a combustion chamber, a gas cooler, a bag filter, an induced draft fan, and a catalytic reactor. The capacity of the test facility was 10 t/day (when 100% ASR was processed). ASR introduced into the melting furnace was gradually dried and preheated in the upper section. Subsequently, combustible waste was thermally decomposed, and pyrolysis gas was discharged from the top of the melting furnace. The pyrolysis gas was transferred to the combustion chamber and was then completely burned. Meanwhile,

incombustible waste and remaining residues descended to the bottom of the melting furnace and melted completely, the heat being generated by burning coke. Finally, molten materials were discharged from the taphole, quenched with water, and magnetically separated into slag and metal. The gas emission control system consisted of a gas cooler, bag filter, and catalytic reactor. The temperature of the melt was about 1,700°C, which allowed smooth delivery of the melt during the test period. The temperature at the outlet of the combustion chamber was 960°C, and the exhaust gas from the stack amounted to 11,000 Nm^3/t dry weight. Furthermore, the fact that the CO concentration of the exhaust gas was only 4 ppm proved that complete combustion of the pyrolysis gas generated from the melting furnace was possible.

Table 3 shows the behavior of dioxin-related compounds and BFRs. Although the ASR contained 30 mg/t of PBDD/DFs, this amount was reduced to 1.3 μg/t at the outlet of the combustion chamber, the indication being that the gasification and combustion process had decomposed more than 99.99% of the PBDD/DFs. The cooling process showed almost no resynthesis, unlike the behavior of PCDD/DFs. With a total emission at 14 μg/t, 99.9% of the input PBDD/DFs had been decomposed, with 79% of the total emissions accounted for by fly ash. No PBDD/DFs were detected in the slag or metals; similarly, mono-BrPCDD/DFs were not detected in the exhaust gas or slag. Because mono-BrPCDD/DFs were detected in the fly ash, resynthesis during the cooling process may have taken place. PBDEs at the outlet of the combustion chamber amounted to 22 μg/t, the indication being that more than 99.99999% of the PBDEs input to the melting furnace had been decomposed by the gasification and combustion process. The total emission was 170 μg/t, which means that 99.9999% of the brominated diphenyl ethers input to the melting furnace were decomposed, with 71% of the emission discharged as fly ash. The TBBP-A at the outlet of the combustion chamber was 156 μg/t, the indication being that more than 99.99% of the input TBBP-A had been decomposed by the gasification and combustion process. The total emission of TBBP-A was 350 μg/t, which means that more than 99.99% of the input TBBP-A had been decomposed, with about 89% of the emission discharged as exhaust gas. Based on these results, the melting method was proven to be effective in the decomposition of POPs such as PBDEs, TBBP-A, PBDD/DFs, and PCBs.

Formation of polybrominated dibenzofurans (PBDFs) was clearly found by Kajiwara et al. [25] in the flame-retarded plastics. They investigated the high-impact polystyrene and DecaBDE samples and found that the PBDF concentration increased by about 40 times after 1 week of exposure under natural sunlight conditions, with a concomitant decrease in BDE 209. Formation mechanism of brominated dioxins and dibenzofurans should be taken care, and more studies are necessary.

Table 3 Behavior of BFRs and PBDD/DFs in ASR treatment [24]

	Charged	Outlet of each section		Emissions				
	ASR	Exhaust gas at outlet of combustion chamber	Exhaust gas at outlet of gas cooler	Exhaust gas at outlet of catalytic reactor	Fly ash at bag filter	Slag discharged from melting furnace	Metals discharged from melting furnace	Total emissions (Unit: ug/ton of waste)
PCDDs/DFs	970	3.1	–	1.7	4,300	71	140	4,500
Co-PCBs	30,000	1.9	–	1.8	200	7.1	3.7	210
PBDDs/DFs	30,000	1.3	ND	2.2	11	ND	ND	14
MoBrPCDDs/DFs	ND	ND	–	ND	1,900	ND	4.0	1,900
Brominated diphenyl ethers	310,000,000	22	270	ND	120	26	25	170
Tetrabromobisphenol A	15,000,000	156	150	310	13	18	6.1	350
PCBs	270,000	17	–	29	960	24	18	1,000

ND not detected, – not measured

6 Hierarchy of Persistent Chemical Management

The global consensus on the basic concept of hierarchical measures for solid waste management is (1) reduction, (2) reuse, (3) recycling, and (4) proper treatment and disposal. This concept also makes it possible to develop an in-depth discussion on management measures, especially for hazardous wastes and persistent chemicals that have high potential to cause environmental damage. The concept of "clean, cycle, and control" represents what measures should be taken to control persistent chemical substances [26]. This concept means that the use of hazardous chemicals should be avoided (clean), the principle of recycling (cycle) should be introduced when appropriate alternatives are not found and the material must be used for its effect, and waste from previous use should be decomposed as much as possible and stabilized, the focus being on control of the waste (control).

The use of clean measures is a concept similar to green chemistry, the philosophy of which was first developed in 1990s [27]. It promoted both technological and policy development that encourages design of products and processes that minimize use and generation of hazardous substances. The principles of green chemistry include the following concepts:

- Chemical products should be the ones that possess minimum toxicities to human health and the environment.
- Substances with the lowest toxicity among those with similar functionality should be used as much as possible.

This idea of "green chemistry" is one of the effective measures to realize the clean concept. It can be said that implementation of this concept has brought about a dramatic change to the basic modus operandi of the chemical industry, and similar ideas will hopefully spread to other industries. The clean principle should be applied wherever possible; however, if this is not possible, or in cases where other, other management practices are required, then the second principle of cycle should be adopted.

The basic elements of technologies for control are the following: separation, recovery, reuse and decomposition, and stabilization/solidification. When treating hazardous waste and/or persistent chemicals, the following steps should be taken in the given order: (1) separation/recovery, (2) detoxification, and (3) stabilization. First, it is necessary to design products from the standpoint of their separation, recovery, and reuse. Then, the detoxifying processes, which can remove hazardous substances contained in waste products or waste materials, should be given priority. In other words, use thermochemical treatment such as incineration and melting that bring about essential changes of hazardous characteristics. Thermochemical treatment under proper conditions is particularly effective for various organic compounds, including organochlorine compounds contained in organic materials. This treatment is the ultimate goal of effective technologies for detoxification. For metal-containing wastes, solidification/stabilization technologies should be the

Table 4 Clean, cycle, and control concept and some examples for POPs

	Clean	Cycle	Control
General concept	Avoid the use of hazardous chemicals and use alternatives	When there are no appropriate alternative substances and the use of specified material is essential because of its crucial effect, recycling should be the principle	Control of emission to the environment and the decomposition and stabilization of stock substances and wastes which have been used in the past
PCB	Use of PCB should have been almost completely stopped from the viewpoint of adverse effects on human health and the environment	Recycling used in closed system, but too difficult to control without emission over the whole life cycle	Control of emissions in the stages of repair, demolition, and disposal must be fully enforced. Complete destruction of waste PCB should be carried out
Chlorinated dioxins	Chlorinated dioxins are produced as by-products by chemical or thermal reactions. Therefore, avoidance of unintentional formation of chlorinated dioxins is a first priority	No way for cyclical use. Environmental cycles should be avoided and reduced	Control of the environmental emissions should be done by the Best Demonstrated Available Technology (BDAT) measures. Additional monitoring on food to have minimum human exposure is also one kind of control measure
BFRs	PeBDEs and OBDEs received a risk assessment as toxic, and their production was stopped internationally. DBDE is still produced and used in various products	Part of BFRs is reused and recycled. The regulation on PBDEs exempts recycling activities	Continue to develop clean and control measures for BFRs

principal effective disposal process, because metals are not subject to decomposition.

Table 4 shows some examples of the 3C concept (clean, cycle, and control principle), with POPs as an example. The first priority for intentionally produced POPs is implementation of the clean concept. This concept was applied to PCBs as soon as their toxicity and environmental transport were confirmed, their manufacturing was stopped, and their use was limited to closed systems only. At the same time, the Stockholm Convention on POPs designated PeBDEs and OBDEs, which are used in BFRs, as substances targeted for reduced use. In other words, the basic principle here is that toxic substances and substances with the potential for global environmental transport should not be used, and hazardous chemicals that were used in the past without an awareness of their toxicity should no longer be used. The second priority is that unintentionally produced POPs like dioxins should be regulated via the clean concept. Their formation and emission

and transport into the environment should be avoided, and the control concept should be applied to the control of their exposure to humans or to the environment. The third priority is that the cycle concept be adopted in some cases; targeted objects are useful, but metals like mercury and lead are toxic. Among the POPs, it is reasonable that the recycling process for only PBDEs is exempt from regulation.

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Analysis of Dioxin and Dioxin-Like Compounds

Eric J. Reiner

This chapter is dedicated to Otto Hutzinger a pioneer in the analysis of dioxin and an inspiration to environmental analytical chemists.

Abstract The analysis of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls, and other related dioxin-like compounds requires complex sample preparation and analytical procedures using highly sensitive and selective state-of-the-art instrumentation to meet very stringent data quality objectives. The analytical procedures (extraction, sample preparation), instrumentation (chromatographic separation and detection by mass spectrometry), and screening techniques for the determination of dioxins, furans, dioxin-like polychlorinated biphenyls, and related compounds with a focus on new approaches and alternate techniques to standard regulatory methods are reviewed.

Keywords Analysis, Dioxin-like polychlorinated biphenyls, dlPCBs, Extraction, Fast gas chromatography, Fast GC, Isotope dilution, Mass spectrometry, PCDD, PCDF, Polychlorinated dibenzofurans, Polychlorinated dibenzo-*p*-dioxins, QA/QC, Quality assurance/quality control, Review, Sample preparation, Screening techniques

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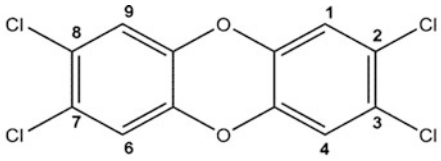
Contents

1 Introduction

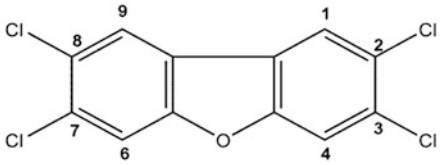
Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are considered among the most toxic groups of chemicals known to man [1, 2]. They are listed in the Stockholm Convention on Persistent Organic Pollutants (POPs) [3] for reduction or elimination. PCDD/PCDFs, as shown in Fig. 1, are planar molecules with two benzene rings connected by either one (furan) or two oxygens (dioxin). They can contain from one to eight chlorines forming a possible 75 different dioxins and 135 different furan congeners. This group of 210 chlorinated compounds is typically called "dioxin" and will be referred to as such in this paper. Congeners containing chlorines in the 2,3,7,8 positions (17 of 210) exhibit dioxin-like toxicity as a result of their preferential binding with the aryl hydrocarbon receptor (AhR) [4]. Toxic effects of dioxins include weight loss, reproductive disorders [5], immune impairment [5], and cancer [6, 7]. A number of other compounds including polychlorinated biphenyls (PCBs) with one or no chlorines in the ortho positions (2 or 2′, 6 or 6′) [8] and some polychlorinated naphthalenes (PCNs) [9, 10] also exhibit dioxin-like character and are classified as dioxin-like compounds (DLCs). DLCs work through a common mechanism, by deregulating the expression of key genes resulting from their interaction with the AhR. As a result, their dioxin relative potency can be compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-T_4CDD), the most toxic dioxin congener [11–13]. The toxic equivalent factor (TEF) is a value assigned to a specific DLC comparing its potency to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [14, 15]. The sum of the concentration of each dioxin-like compound multiplied by its TEF is the toxic equivalent quantity (TEQ) and is the relative amount of DLCs converted 2,3,7,8-T_4CDD equivalents.

$$\mathrm{TEQ} = \Sigma[\mathrm{PCDD}_i \times \mathrm{TEF}_i] + \Sigma\left[\mathrm{PCDF}_j \times \mathrm{TEF}_j\right] + \Sigma[\mathrm{PCB}_k \times \mathrm{TEF}_k] + \Sigma[\mathrm{DLC}_l \times \mathrm{TEF}_l]$$

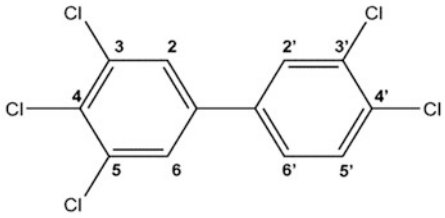
A number of TEF schemes have been reported in the past 30 years. Internationally, accepted TEF schemes have been developed and since 1994 are reevaluated every



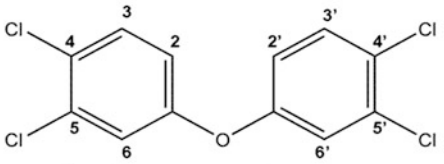
2,3,7,8-tetrachlorodibenzo-p-dioxin



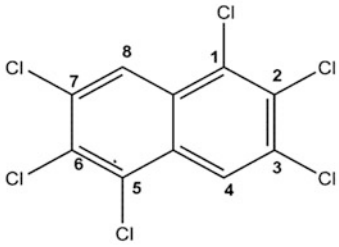
2,3,7,8-tetrachlorodibenzofuran



3,3',4,4',5-pentachlorobiphenyl (PCB-126)



3,3',4,4'-tetrachlorodiphenlyether



1,2,3,5,6,7-tetrachloronaphthalene (PCN-67)

Fig. 1 Structures of dioxin-like compounds: polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls, polychlorinated diphenylethers, and polychlorinated naphthalenes

5–10 years by an expert committee designated by the World Health Organization (WHO) [14, 15]. TEF values have also been developed for PCBs and brominated dioxins [14–16] (see Table 1). For other DLC compounds like PCNs, relative

Table 1 Comparison of various toxic equivalent factors (TEF) schemes

	Eadon 1982	Ontario 1984	Germany 1985	California 1986	USEPA 1987	Nordic 1988	NATO-I 1989	WHO 1994	WHO 1998	WHO 2005
PCDDs										
2,3,7,8-TCDD	1	1	1	1	1	1	1		1	1
1,2,3,7,8-PeCDD	1	0.1	0.1	1	0.5	0.5	0.5		1	1
1,2,3,4,7,8-HxCDD	0.03	0.1	0.1	0.03	0.04	0.1	0.1		0.1	0.1
1,2,3,6,7,8-HxCDD	0.03	0.1	0.1	0.03	0.04	0.1	0.1		0.1	0.1
1,2,3,7,8,9-HxCDD	0.03	0.1	0.1	0.03	0.04	0.1	0.1		0.1	0.1
1,2,3,4,6,7,8-HpCDD	0	0.01	0.01	0.03	0.001	0.01	0.01		0.01	0.01
1,2,3,4,6,7,8,9-OCDD	0	0.0001	0.001	0	0	0.001	0.001		0.0001	0.0003
PCDFs										
2,3,7,8-TCDF	0.33	0.5	0.1	1	0.1	0.1	0.1		0.1	0.1
1,2,3,7,8-PeCDF	0.33	0.5	0.1	1	0.1	0.01	0.05		0.05	0.03
2,3,4,7,8-PeCDF	0.33	0.5	0.1	1	0.1	0.5	0.5		0.5	0.3
1,2,3,4,7,8-HxCDF	0.01	0.1	0.01	0.03	0.01	0.1	0.1		0.1	0.1
1,2,3,6,7,8-HxCDF	0.01	0.1	0.01	0.03	0.01	0.1	0.1		0.1	0.1
1,2,3,7,8,9-HxCDF	0.01	0.1	0.01	0.03	0.01	0.1	0.1		0.1	0.1
2,3,4,6,7,8-HxCDF	0.01	0.1	0.01	0.03	0.01	0.1	0.1		0.1	0.1
1,2,3,4,6,7,8-HpCDF	0	0.01	0.01	0.03	0.001	0.01	0.01		0.01	0.01
1,2,3,4,7,8,9-HpCDF	0	0.01	0.01	0.03	0.001	0.01	0.01		0.01	0.01
1,2,3,4,6,7,8,9-OCDF	0	0.0001	0	0	0	0.001	0.001		0.0001	0.0003
dl-PCBs										
PCB-077 (3,3′,4,4′-TCB)								0.0005	0.0001	0.0001
PCB-081 (3,4,4′,5-TCB)								0	0.0001	0.0003
PCB-105 (2,3,3′,4,4′-PeCB)								0.0001	0.0001	0.00003
PCB-114 (2,3,4,4′,5-PeCB)								0.0005	0.0005	0.00003
PCB-118 (2,3′,4,4′,5-PeCB)								0.0001	0.0001	0.00003
PCB-123 (2′,3,4,4′,5-PeCB)								0.0001	0.0001	0.00003
PCB-126 (3,3′,4,4′,5-PeCB)								0.1	0.1	0.1
PCB-156 (2,3,3′,4,4′,5-HxCB)								0.0005	0.0005	0.00003
PCB-157 (2,3,3′,4,4′,5′-HxCB)								0.0005	0.0005	0.00003
PCB-167 (2,3′,4,4′,5,5′-HxCB)								0.00001	0.00001	0.00003
PCB-169 (3,3′,4,4′,5,5′-HxCB)								0.01	0.01	0.03
PCB-189 (2,3,3′,4,4′,5,5′-HpCB)								0.0001	0.0001	0.00003

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potenticies [10] can be used for TEQ determination. Most laboratories now only report the seventeen 2,3,7,8-substituted congeners and TEQ values. TEQ values make it easier to compare results between samples, but congener-specific pattern information is lost making it more difficult to identify or indicate sources of contamination. For source tracking, full congener analysis can help identify sources of contamination. This procedure can be time-consuming as the remaining 193 non-2,3,7,8-substituted congeners must be quantified and their relative concentrations recorded [18–21]. Monitoring the impurities formed along with dioxin in samples can be used to track and apportion sources. Parette et al. [22] used tetrachlorodibenzothiopehene formed from the thiophenol impurity in Agent Orange manufacturing to track specific sources of dioxin contamination. The focus of this chapter is on PCDD/PCDFs and dlPCBs. A brief summary of the analytical chemistry of associated DLCs is provided at the end of this chapter.

The goal of any analytical method is to quantitatively and selectively as possible extract target analytes from the sample matrix while removing the desired analytes from the bulk matrix and potential interferences and to detect the analytes of interest using an appropriate selective and sensitive detector. The analysis of dioxin is one of the most challenging procedures in analytical chemistry. Due to the highly toxic nature of dioxin, method detection limits are up to three orders of magnitude lower than for most other organic contaminant Stockholm POPs in order to meet the strict regulatory limits and guidelines ($<$1 ppt (picogram per gram for food based on fat or tissue) per congener [23, 24], $\leq$1 ppt for soils/sediments, $\leq$10 ppq (picograms per liter) for aqueous samples, and less than 1 pg/m^3 for air samples); concentration factors of 10^6–10^9 are needed to meet these detection limits. Levels of many interfering compounds like PCBs and polychlorinated diphenyl ethers (PCDEs) must be removed so that extracts can be concentrated to the very small volumes needed to meet the very low detection limits required to meet regulatory limits or guidelines for the protection of wildlife and human health. For aqueous samples, the dioxin in 1 L of sample is extracted, cleaned, and concentrated to an extract of about 20 μL. The classical method uses a three-stage silica, alumina, and carbon cleanup, and in order to achieve accurate quantification, isotope dilution mass spectrometry (IDMS) with ^{13}C-labeled surrogate standards is necessary. Carbon-13 labeled analogs of dioxins, furans, and dlPCBs are added to the sample prior to extraction to account for analyte loss and instrument variability. Gas chromatography (magnetic sector)-high-resolution mass spectrometry (GC-HRMS) is typically used for detection and quantification because of its superior sensitivity and selectivity.

There are a number of regulatory methods available for the analysis of dioxin and dioxin-like compounds. A number of these methods are summarized in Table 2. Specific details regarding analytical standards, labware, instrumentation, procedures, calibration, and quality control are outlined within these methods.

Table 2 Various analytical methods for dioxin-like compounds

Method	Method description/analytes	Source/year of publication
US EPA 23	Determination of 2,3,7,8-substituted dioxins and furans with congener group totals in incinerator stack gasses by isotope dilution (ID) – GC-HRMS	US EPA 1995
US EPA 1613b	Determination of 2,3,7,8-substituted dioxins and furans with congener group totals in water and wastewater by GC-ID HRMS	US EPA 1994
US EPA 8290 (SW-846)	Seventeen 2,3,7,8-substituted dioxins and furans with congener group totals in materials and waste. Uses isotope dilution – GC-HRMS	US EPA 1994a
US EPA TO-9A	Compendium of methods for the determination of toxic organic compounds in ambient air	US EPA 1999
	Second edition compendium method TO-9A determination of polychlorinated, polybrominated, and brominated/chlorinated dibenzo-*p*-dioxins and dibenzofurans in ambient air	
US EPA 1668a	Determination of all 209 PCB congeners. 12 WHO dioxin-like PCBs by GC-HRMS, the remaining 197 by GC-MS	US EPA 1999
ISO 17025	General requirements for the competence of testing and calibration laboratories	ISO 2005
ISO 17858	Determination of 12 WHO dioxin-like PCBs in environmental matrices by GC-ID HRMS	ISO 2006
ISO 18073	Determination of seventeen 2,3,7,8-substituted dioxins and furans with congener group totals in water and wastewater by GC-ID HRMS	ISO 2004
ISO 16780	Determination of polychlorinated naphthalenes (PCN) – method using gas chromatography (GC) and mass spectrometry (MS)	ISO 2015
MOE E3418	Determination of 2,3,7,8-substituted dioxins and furans including congener group totals and 12 WHO dioxin-like PCBs by GC-ID HRMS	MOE 2012
MOE E3431	Determination of polychlorinated naphthalenes (PCNs) in environmental matrices by GC-HRMS	MOE 2012
EU EN 1948	Seventeen 2,3,7,8-substituted dioxins and furans and congener group totals in stationary sources by isotope dilution – GC-HRMS	European Standard 1997
ENV CAN 1/RM/19	Seventeen 2,3,7,8-substituted dioxins and furans and congener group totals in pulp and paper effluents by isotope dilution – GC-HRMS	Environment Canada 1992
JIS K0311	Seventeen 2,3,7,8-substituted dioxins and furans including congener group totals in incinerator stack gasses by isotope dilution – GC-HRMS	JIS 1999a
JIS K0312	Seventeen 2,3,7,8-substituted dioxins and furans including congener group totals in wastewater by isotope dilution – GC-HRMS	JIS 1999b

Note: US EPA methods available from www.usepa.org, ISO methods from www.ISO.org, and MOECC methods from LaboratoryServicesBranch@Ontario.ca

2 Extraction

Quantitative extraction of the analytes from the bulk matrix is critical to obtain quality data. A wide variety of extraction techniques and systems have been used for the extraction of DLCs. Sonication; Soxhlet extraction; pressurized fluid extraction (PLE – also known as accelerated solvent extraction (ASE)); microwave-assisted extraction (MAE), also called microwave-assisted solvent extraction (MASE); solid-phase extraction (SPE); liquid/liquid extraction; and supercritical fluid extraction (SFE) have all been used to extract dioxin and DLCs from bulk matrix. Passive sampling devices such as semipermeable membrane devices, polymer (e.g., polyethylene and polydimethylsiloxane) passive samplers, sorptive stir bars, window films, and tree bark have also been used to extract dioxin-like compounds from the bulk sample matrix. The hydrophobic nature of DLCs makes them very soluable in nonpolar organic solvents. Aromatic solvents like benzene or toluene are preferred, but require extensive safety precautions due to their toxicity and carcinogenic nature. Benzene is a controlled substance in many countries and is now rarely used except for analytical standard preparation. Other solvents like hexane and/or dichloromethane are also used, but are not as effective as toluene which is the solvent of choice for most applications. These extraction methods are detailed in a number of publications [25–28].

The extraction of solid matrices including soil, sediment, sludge, biological tissue, and vegetation has classically been done by Soxhlet extraction. Soxhlet extraction is simpler and less expensive, but more labor intensive when compared to other newer procedures, and is typically the recommended or required procedures in most regulatory methods. Wet samples (sediments, biota, and vegetation) can be Soxhlet extracted with a Dean-Stark device, which separates the water from less dense nonpolar solvent. The method allows the drying step to be skipped. The water is collected in an alternate receiving vessel. The amount of water can be determined volumetrically or gravimetrically enabling moisture content of the pre-extracted sample to be determined [29, 30]. Sonication and agitation – shaking in a paint shaker – have also been used. They can be less efficient than Soxhlet but are much faster.

Automated extraction methods like PLE and MAE can run unattended. PLE is a pressurized filtration method that subjects the sample to heat and elevated pressure [31–33] which can enhance extraction efficiency over room temperature procedures. MAE [34–36] uses microwave energy to produce heat resulting increased pressure for enhancing extraction efficiency. Microwave-assisted extraction requires the use of a polar solvent or a solvent mixture containing a polar solvent like acetone or methanol to convert microwave energy to heat. Because a polar solvent is used, samples do not have to be dried prior to extraction. Both PLE and MAE can be used to reduce extraction times over the classical Soxhlet and sonification procedures.

A number of PLE methods have been developed where column packing materials are inserted in a modified PLE cell so that extraction and sample cleanup can

be completed in the same step [37–39]. This can result in very quick extraction and preparation of relatively clean samples. Highly concentrated and complex samples can severely contaminate or overload the system, and therefore prior knowledge of levels of dioxin and other coextractables in the samples is important when using this method. Biological samples and wet sediment/soil samples can be extracted using matrix solid-phase dispersion (MSPD). Wet samples are ground and mixed with a solid dispersant (e.g., silica, diatomaceous earth, sodium sulfate) and placed in a Soxhlet or PLE cell where the analytes are extracted, while the water remains on the dispersant. Extreme care must be taken in determining moisture content as this can result in significant variability or bias if the sample being analyzed is not homogeneous and the resulting relative dry weights are not calculated correctly. This is also the case for biological samples. Dioxin and other POPs partition into the lipid tissue. Care must be taken to ensure that the sample is homogenized properly to minimize variability and bias. Results for biological samples can be reported on wet-weight or lipid-weight basis. If lipid levels vary greatly between organisms, lipid corrected data can provide better results for comparison [40].

Supercritical fluid extraction (SFE) using pressurized CO_2 [41] has been used to extract dioxins from soils and sediments [42] and marine biota [43]. SFE is a solventless extraction procedure that may need only very small amounts of solvent to elute the analytes from the trapping or packing material (e.g., carbon or C_{18}).

The extraction of liquid samples can be challenging, especially if they contain significant amounts of solid particulate material (SPM). Dioxins and other DLCs have log Kow values that range from 4 to 8; therefore, they tend to adsorb to SPM, and extraction schemes must be able to quantitatively remove analytes from the SPM. The classical dioxin extraction method is liquid/liquid extraction (LLE). The LLE method is very labor intensive; therefore, aqueous and other liquid samples including biological fluids are now often extracted using solid-phase extraction (SPE), a technique that uses a solid stationary phase like C_{18} or Amberlite XAD-2 resin in an extraction cartridge or disk to extract nonpolar dioxin-like compounds and pass through polar or slightly compounds. The classical dioxin procedure for aqueous samples is to filter the sample to collect the particles. The filtered portion is then extracted using liquid/liquid or solid-phase extraction, and the filter is extracted by Soxhlet extraction or PLE. If the amount of SPM is relatively low, e.g., below 2 g/L, direct extraction of samples by SPE is possible [44–46]. The particles are trapped on top of the extraction disk or column bed. Quantitative elution of the particles and disk can be done in a single step, significantly reducing solvent usage and sample preparation time. Erger et al. [47] have reviewed the various solid phases and uses of SPE disks for samples that contain SPM. The SPE disk extraction procedure can be automated if SPM levels are low [48, 49]. Aqueous samples that contain very small amounts or no visible levels of SPM can also be extracted using stir-bar sorptive extraction followed by thermal desorption [50].

Passive samplers have been used for a number years as integrative samplers to assess long-term time-weighted average levels of contaminants in water and air. Semipermeable membrane devices (SPMDs) were some of the first used [51]. Passive samplers for aqueous media include SPMDs (triolein in a semipermeable

membrane housing), silicone rubber (SR), low-density polyethylene (LDPE) strips, and polydimethylsiloxane (PDMS) [52–58]. If performance reference compounds (PRCs are labeled or other internal standards) are spiked into the samplers, their rate of elimination from the samplers can be used to determine relative sample volumes to estimate contaminant concentrations [55]. Sample volumes with passive samplers are not measured directly but are estimated using lab-based constants and PRC behavior which varies from compound to compound. Passive samplers collect only the dissolved fraction of dioxin, and therefore results do not compare directly to those using the whole-water extraction methods described above. Due to their K_{OW}s, the majority of dioxins are present on the particulate phase. The dissolved phase, however, is the bioavailable fraction, and as a result, passive samplers can be used as surrogates for exposure to dioxin and other compounds with low K_{OW}s. Passive samplers are extracted using the same methods that are used for solid samples: Soxhlet, sonication, and PLE.

Passive samplers used for air sampling are typically polyurethane foam (PUF) plugs housed in a metal canister and can include a type of XAD resin. Other sampler designs have included just the XAD resin in a metal housing [59–61]. Passive air sampling has also been carried out using window films, building wipes [62, 63], and tree bark [64, 65]. Passive samplers do not require electrical power and therefore can be deployed for extended periods of time enabling good detection limits to be achieved.

Ambient air samples are typically sampled using high-volume samplers. Air is passed through an 8½ by 11 in. filter paper to trap the particulate fraction containing penta to octa dioxin/furan congeners followed by a PUF to trap the volative (mono to penta) congeners. Some configurations use XAD sandwiched between 2 PUFs. The filters and PUFs are extracted using Soxhlet or large cell PLE systems. See method US EPA TO-9 or MOECC 3418 for more details.

3 Extract Cleanup

The extraction of dioxin-like compounds with nonpolar solvents or media is not very selective. The highly aggressive extraction methods used with nonpolar solvents or nonpolar media for DLCs co-extract many additional POPs and matrix compounds which can interfere in the analysis and must be removed in the sample preparation process. This requires a comprehensive sample preparation scheme to remove bulk matrix coextractables, eliminate any potential interfering compounds, and retain as much of the analytes of interest as possible while achieving the high concentration factors listed above. The classical dioxin cleanup developed over 30 years ago is still basically the same procedure used today. The method originally developed by Nesterick and Lamparski [66] referred to as the “Dow cleanup” was later modified by Smith and Stallings [67] using a multilayered acid/basic silica column to remove acidic/basic polar compounds and silver nitrate/silica to remove any sulfur compounds, especially any chlorinated dibenzothiophenes. Silver nitrate

is more expensive than mercury or copper but much faster and easier to use and not a significant additional cost to the method. Mercury is toxic and now only typically used in emergency response situations. Sulfur removal is less critical nowadays as most laboratories are using HRMS or tandem mass spectrometry (MS/MS) detection systems.

Column chromatography with various combinations of silica, alumina, Florisil, and carbon adsorbents can be used to remove bulk matrix and interfering compounds and to direct specific analytes into desired fractions [68–73]. Due to the ability to extract a variety of analyte groups together (dioxin, PCBs, PBDEs, PCNs, etc), sample preparation schemes have been developed to combine analytical methods and fractionate extracts to eliminate any potential interfering compounds. For example, activated carbon can separate the planar from the nonplanar compounds. Dioxin and coplanar PCBs and PCNs can be collected in one fraction and the nonplanar compounds (ortho-PCBs, PBDEs, PCDEs, chlorinated pesticides) in another. This eliminates a number of the gas chromatographic coelutions, e.g., PCB77/110 and PCB 87/81, and also splits the PCDE a major interference to PCDFs into separate fractions [74]. The PCDEs can fragment in the mass spectrometer ion source to form PCDFs which can result in a significant bias. They are highly bioaccumulative and can be a major interference to PCDFs in biological samples. In all cases, the use of each adsorbent type must be carefully characterized and calibrated to optimize elution volumes of the analytes of interest while minimizing the contribution of interferences.

Alumina and Florisil are used to remove or separate (e.g., ortho-substituted PCBs) some of the less polar compounds as well as residual lipids not removed by the silica column cleanup. Any combination of silica, carbon, alumina, or Florisil can be used to remove potential interferents. The strategy is to use minimal amounts of packing to reduce contamination and possible irreversible adsorption of analytes on the packing material to achieve the highest analyte recoveries possible. For difficult samples, additional amounts of packing materials and cleanup stages may be needed to produce an extract clean enough to inject on the gas chromatograph. For samples with high levels of lipids, e.g., biological tissue or biosolids, gel permeation chromatography (GPC) [75] can be used as a preliminary cleanup step. Additional amounts of acid silica packing can also be used to remove excess lipids. This option is preferred if sample extracts are not also used to analyze acid labile compounds like some of the brominated flame retardants.

Classical open-column cleanup procedures like the Smith/Stallings method (silica, alumina/Florisil, or carbon) can be very labor intensive. It can 3–5 days extract and cleanup a set of 10–20 samples using manual procedures. Each column eluant is concentrated, typically to dryness. This is one of the most important steps in the analytical scheme as aggressive extract concentration can result in analyte loss, low surrogate recovery, and elevated detection limits.

In order to minimize labor costs, a number of automated or semiautomated systems have been developed. Fluid Management Systems Inc. (FMS – Watertown MA, USA) developed the first automated dioxin sample preparation system, the PowerPrep System, that uses a variety of different disposable prepackaged columns

which allows for the ability to prepare extracts for a single group of compounds (e.g., dioxin, PBDEs) or multiple compound groups or a combination of analyte groups together in a single method [46, 76]. Figure 2 shows the FMS automated sample preparation system. In recent years, other systems have become available from a variety of vendors including LCTech (Dorfen Germany) and Miura Co (Ehime Japan, also available from DSP Systems, Erichem Netherlands). Fayez et al. [78] reported that the cost of preparing dioxin extracts using the FMS system is about the same as conventional manual methods; however, a single analyst can prepare double the number of samples in the same period of time. For larger contract labs that analyze many thousands of samples per year, using assembly line processing and manual methods with disposable labware can be more cost-effective.

Fayez et al. [79] have also used the FMS system to extract and cleanup dioxin, PCBs, PCNs, and BDEs in biological tissue in a single run using PLE extraction with combined manual transfer to the FMS system for cleanup. This method reduces the extraction/sample preparation time to 2–3 days significantly reducing analytical time and costs. Doing the four tests separately using manual methods could take 10 days to extract and prepare. The ortho-substituted PCBs and BDEs are collected by forward elution through the activated carbon column, while dioxin, coplanar PCBs, and PCNs are collected in a reverse elution fraction. Focant et al. [80] were able to interface a PLE with the FMS system to automatically extract and cleanup a set of food samples ready to be analyzed in about 10 h.

These automated systems can work very well with samples like biological tissues and food where the matrix is relatively constant and analyte levels do not vary significantly. In cases where high concentrations are expected or there is heavy matrix loading, manual methods may be preferred so that the automated systems are not contaminated to the point that background levels interfere with detection limits.

Cape Technologies (South Portland ME, USA) have developed a semiautomated method using disposable glass columns. The system (Fig. 3) is pressurized with nitrogen gas. A multi-analyte compound group method (dioxin, PCBs, PBDEs, and PCNs) similar to that developed for the FMS system was developed by Yang et al. [77, 81, 82]. The major advantage of the Cape system is that it does not require power, and because the sample extract only makes contact with disposable labware, no rinse cycles and labware cleaning are needed making this a very cost-effective sample preparation method. Because the sample never touches any reusable labware, cross contamination from high-level samples is minimized. Some high production contract labs use disposable labware methods similar to the Cape procedure, but find it more cost-effective to purchase reagents and adsorbents in bulk and pack their own columns.

There have been a number of publications reporting the extraction and cleanup using an ASE or modified ASE or PLE cell. The samples are typically mixed with some type of matrix dispersant like diatomaceous earth or hydromatrix and are placed above a series of column packings like silica or carbon in the extraction cell allowing extraction and sample cleanup to be carried out simultaneously [38, 39, 83–85].

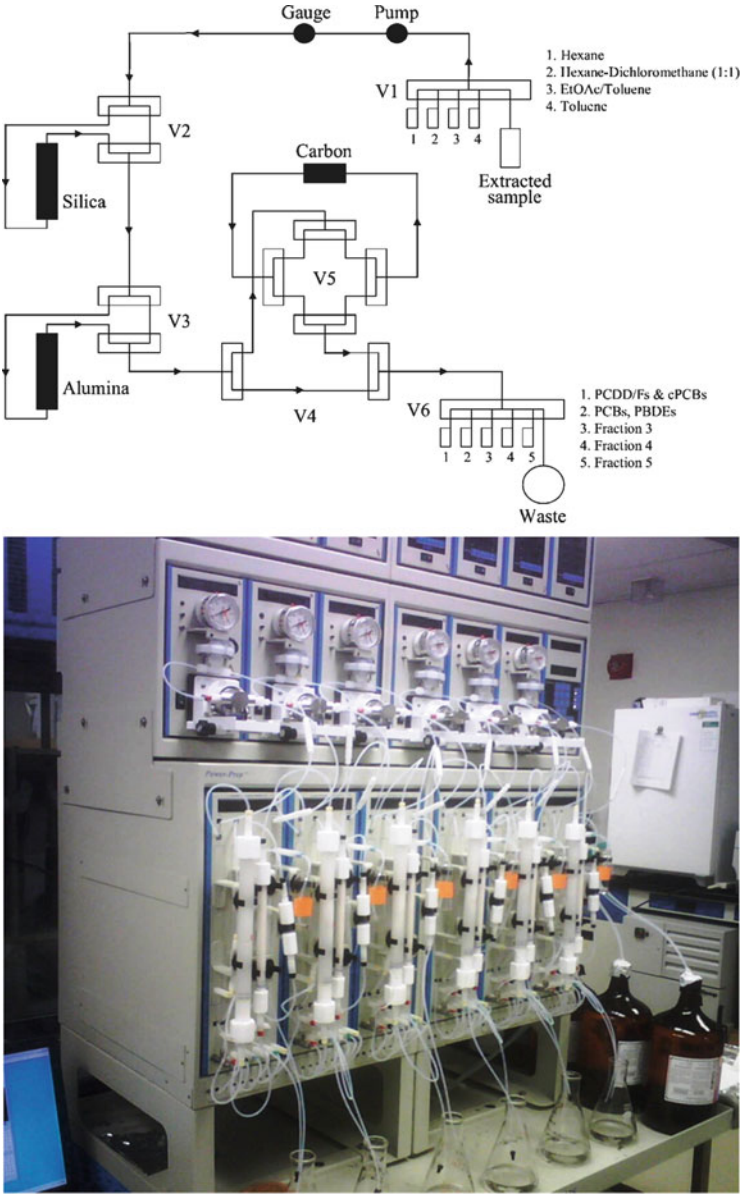

Fig. 2 (**a**) Schematic representation of an automated cleanup system. Reproduced with permission from [76] of GC-MS, (**b**) FMS PowerPrep System

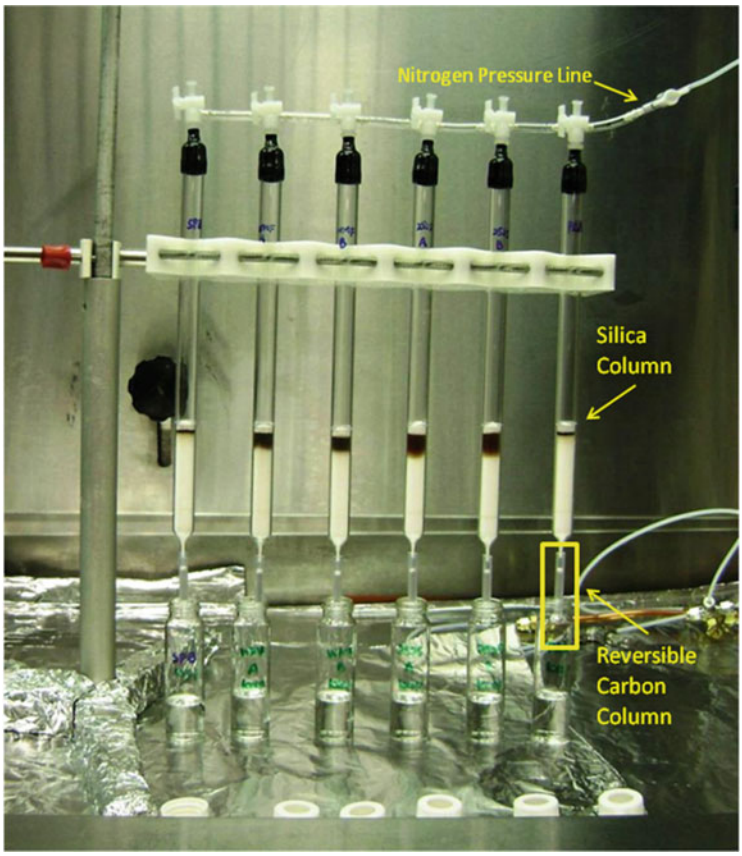

Fig. 3 Cape Technologies Disposable Sample Preparation System (www.Cape-Tech.com)

4 Chromatographic Separation

Dioxins, furans, and PCBs contain many isomers or congeners of similar structure, and as a result of the symmetrical nature of these compounds, the mass spectra of their isomers (e.g., tetra dioxins) are essentially identical. In addition, congeners of higher degrees of halogenation can fragment in the mass spectrometer to form interfering ions at mass-to-charge ratios of their lower halogenated homologues. Because mass spectrometers cannot separate isobaric compounds, it is important that the toxic compounds be separated from the nontoxic ones either physically (by placing them in separate fractions) or chromatographically, especially if accurate TEQ values are required.

The majority of dioxin and furan congeners are not considered toxic, and therefore a chromatographic column or combination of columns able to uniquely separate the 17 toxic congeners from one another as well as the remaining

193 nontoxic congeners is required. Coelutions with nontoxic congeners or other interferences can cause biased results. PCBs can interfere with dioxins, and PBDEs can interfere with PCBs [86]. The Wellington Reference and Handling Guide [87] provides information on a variety of halogenated compounds including exact masses and theoretical isotope ratios that can be used to determine potential interferences.

There currently is no single GC column that can uniquely resolve all of the toxic dioxin congeners from the other 193 nontoxic dioxins or other interfering compounds, and therefore, samples must be analyzed on separate GC phases, or sample extracts must be fractionated in a way that interfering compounds are split into different fractions.

There have been a number of attempts to develop a dioxin-specific column that can separate the seventeen 2,3,7,8-substituted congeners. Many of these attempts have resulted in a column with lower numbers of coelutions than the standard 5% phenyl-methyl phases. DB-dioxin [88] was the first dioxin-specific column developed. Rtx-dioxin-2 [89–91] and BPX-DXN [92] are also dioxin-specific GC columns. A number of reviews have compared the separation of dioxin on 5% phenyl-methyl phases to a number of other polar and analyte-specific phases [93–95]. Table 3 compares the ability of a variety of GC columns to separate each of the seventeen 2,3,7,8-substituted dioxins. None of the columns currently available can uniquely separate all the toxic congeners, and therefore in most regulatory methods, the 5% diphenyl-dimethylpolysiloxane column is still used in conjunction with the DB-225 (50% cyanopropylphenyl-dimethylpolysiloxane). A lower bleed version of the 5% diphenyl-dimethylpolysiloxane column was developed by J&W in the early 1990s. The elution pattern of this phenyl arylene polymer is slightly different from the standard 5% phenyl-methyl columns. Abad et al. [96] and Fishman et al. [95] have reported the relative retention times and coelutions for all of 2,3,7,8-substituted dioxins and furans on these "5" phase-ms columns. The 5-MS column can uniquely separate 2,3,7,8-T_4DCF from the other T_4DCFs eliminating the need for secondary confirmation of 2,3,7,8-T_4DCF. The standard GC columns typically used for confirmation for dioxin analysis completed on a 5% phenyl phase are the DB-225 or SP-2331 columns. Both columns are polar, have very low-temperature maximums, and can suffer from significant bleed. A number of liquid crystal columns have been developed for dioxin analysis [97, 98]. These columns can have very high resolving powers for planar compounds and therefore are very selective to dioxins and other dioxin-like compounds. Unfortunately, they typically have low-temperature maximums. A variety of ionic liquid columns have been developed recently. They have higher temperature limits than the liquid crystal columns but are not as stable as conventional cross-linked columns. Do et al. [99, 100] have reviewed the various column combinations. The combination of a 5-MS and SLB-IL61 column will separate all of the 2,3,7,8-substituted congeners and is an alternative combination to the classical 5% diphenyl-dimethylpolysiloxane and 50% cyanopropylphenyl-dimethylpolysiloxane pair.

The GC-HRMS analysis of dioxin using a conventional 5% phenyl-dimethyl, 60 m, 0.25 mm, 0.25 μm, and GC column is about 50 min long. Prior to analyzing

Table 3 Isomer-specific separation of 2,3,7,8-substituted PCDD/Fs on a variety of stationary phases

Congeners	DB-XLB	βDEXcst	LC-50	SLB-IL61	SLB-IL76	SLB-IL111	Equity-5	DB-5 ms	VF-5 ms	VF-Xms	DB-1	DB-5	DB-17	DB-210	DB-225	CPS-1	SP-2331	CP-Sil 88	Smectic	RTX Dioxin2	BPX-DXN	5Sil MS
2,3,7,8-TCDF	−−	−−	−−	++	−−	++	−−	+−	++	++	−−	−−	++	+−	++	−−	+−	+−	−−	+−	+−	+−
1,2,3,7,8-PeCDF	++	++	−−	+−	−−	−−	+−	++	++	++	+−	+−	−−	−−	−−	−−	−−	−−	++	++	++	++
2,3,4,7,8-PeCDF	−−	+−	−−	++	++	++	−−	−−	−−	−−	−−	−−	++	++	++	+−	++	++	−−	−−	−−	−−
1,2,3,4,7,8-HxCDF	++	+−	+−	−−	−−	++	−−	++	++	++	−−	−−	+−	−−	+−	−−	−−	−−	++	+−	+−	++
1,2,3,6,7,8-HxCDF	++	+−	+−	++	++	+−	+−	++	++	++	+−	+−	−−	+−	−−	+−	+−	+−	++	+−	++	++
2,3,4,6,7,8-HxCDF	++	+−	++	++	++	++	+−	−−	−−	+−	−−	+−	+−	++	−−	++	++	++	−−	−−	−−	−−
1,2,3,7,8,9-HxCDF	++	−−	++	++	++	++	+−	−−	−−	+−	+−	+−	++	−−	++	++	++	++	++	−−	−−	+−
1,2,3,4,6,7,8-HpCDF	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
1,2,3,4,7,8,9-HpCDF	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
OCDF	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
2,3,7,8-TCDD	+−	−−	++	++	−−	−−	++	++	++	++	+−	++	−−	−−	+−	−−	++	+−	++	+−	+−	++
1,2,3,7,8-PeCDD	−−	−−	++	++	−−	−−	++	+−	++	++	+−	++	+−	−−	−−	++	++	++	++	−−	+−	++
1,2,3,4,7,8-HxCDD	++	−−	−−	++	++	++	+−	+−	++	++	+−	++	++	+−	++	++	++	++	−−	+−	++	++
1,2,3,6,7,8-HxCDD	+−	−−	−−	++	++	++	+−	+−	++	++	+−	+−	++	++	++	++	++	++	−−	+−	++	++
1,2,3,7,8,9-HxCDD	++	++	++	++	++	++	−−	+−	+−	++	−−	−−	++	++	++	++	++	++	++	++	++	++
1,2,3,4,6,7,8-HpCDD	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
OCDD	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

+ +: baseline separation or at least 10% valley. Peak resolution of $R > 1$
+ −: quantifiable result (separation that allows peak resolution of $R \sim 0.8$)
− −: coelution or interference present. Maximum possible concentration
Data compiled from references [93–95, 99, 100]

samples, the instrument must be tuned to 10,000 resolutions and then calibrated. A resolving power (ΔM/M) of 10,000 is the best compromise between selectivity (eliminating most interfering compounds that are not removed by sample preparation) and sensitivity on a magnetic sector instrument. A series of quality control (QC) samples include a column performance check containing the nearest eluting neighbors of 2,3,7,8-TCDD, low-level standard (sensitivity check), continuing calibration standard, laboratory blank, spiked sample or certified reference material (CRM), and duplicate sample. The setup procedure can take 5 h or more. Continuing calibration methods are used to reduce analytical run times because running a five-point calibration curve can add up to another 4 h to the analytical run. Even when using the continuing calibration procedure, only about 15–18 samples can be analyzed in a single day.

If columns of smaller inner diameters and thinner films (inner diameter <0.20 mm and film thickness <0.18 μm, i.e., microbore columns) are used, a technique called fast GC can significantly reduce analysis times. If the phase ratio (the ratio of film thickness to column inner diameter) remains constant, the relative retention times do not change. As the inner diameter and phase thickness are reduced and their ratio is kept constant, retention times decrease, and the chromatoraphic peaks get narrower and taller. Microbore columns have much lower height equivalent theoretical plates (HETP) than classical columns resulting in significantly increased theoretical plates per meter, enabling significantly shorter columns to be used. Microbore columns require higher column head pressures, flow rates, and temperature ramp rates, which results in reduction of analytical run times by up to 50% and an increase of peak heights of up to a factor of five [101–105].

Multidimensional gas chromatographic techniques have been used for a number of years for the analysis of dioxin and related compounds. Comprehensive multidimensional gas chromatograph or GCxGC is an emerging technique that continues to gain interest. It can enhance sensitivity and reduce analysis times and has been used to analyze dioxins and PCBs [106–114]. GCxGC is carried out using two GC columns of different stationary phases that are connected through a device called a modulator. The modulator traps the analytes eluting from the first (primary) column, compresses their column plug, and then reinjects these small plugs or slices into the second (secondary) column where further separation occurs by a different mechanism that happens in the primary column [110]. Figure 4a shows a schematic of a comprehensive GCxGC system. The modulation process produces much narrower and taller chromatographic peaks which can increase signal-to-noise ratios and sensitivity by up to an order of magnitude. Fig. 4b depicts the procedure, whereas peak that is not completely separated on the primary column (X + Y) is cut into "slices" by the modulator and can be separated on the secondary column as a result the different stationary phase. The slices are small very narrow packets of ions (Fig. 4b) where each packet results from a separate modulation cycle – P_M. The slices can be reconstructed as either a three-dimensional plot or a contour plot. Columns of distinctly different phases (depending on temperature compatibility) can be used resulting in significantly enhanced peak capacity. If the main process of separation for each column is different (e.g., boiling point, polarity, shape

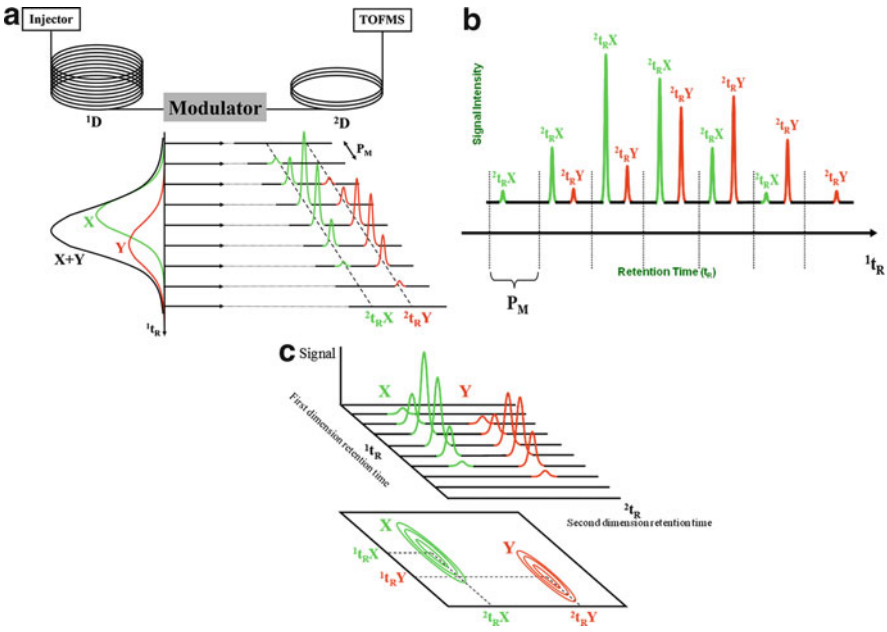

Fig. 4 Schematic of the column coupling in the GCxGC-TOFMS apparatus. (**a**) The modulator allows rapid sampling of the analytes eluting from the first-dimension GC (1D) and reinjection into 2D. The modulation process is illustrated for two overlapping compounds (X and Y) eluting from 1D at a defined first-dimension retention time $^{1}t_R$. As the modulation process occurs during a defined modulation period P_m, narrow bands of sampled analytes enter 2D and appear to have different second-dimension retention times $^{2}t_R(X)$ and $^{2}t_R(Y)$. (**b**) Raw data signal as recorded by the TOFMS through the entire two-dimensional separation process. (**c**) Construction of a two-dimensional contour plot from the high-speed secondary chromatograms obtained in (**b**), in which similar signal intensities are connected by the contour lines. Reproduced with permission from [106]

selection), orthogonal (optimal) separation can be achieved. Orthogonal separations typically result in separation of isomers in bands at an angle of about 45°. The peak capacity in an orthogonal GCxGC system is the product of the peak capacity of the primary and secondary columns and can be typically in the order of 1,000 (approximately 50×20) (first dimension (30 M, 0.25, 0.25) $\times$ second dimension (2 M, 0.10, 0.10)) [115]. The major challenge of this technique is obtaining enough data points across the very narrow peaks produced in the modulation process. GCxGC peaks are in the order of 400 ms wide. In order to accurately define a second-dimension GC peak (7–10 measurements), scan rates of greater than 25 Hz are required. Figure 5 exhibits the power of GCxGC showing a chromatogram of a sediment sample where many analytes can be measured in a single run. In many areas of the chromatogram, there would be multiple coelutions if the extract was analyzed in by regular single-dimensional chromatography.

Electron-capture detectors (ECD) have been used in GCxGC applications because of the significantly enhance separation enables much simpler and less

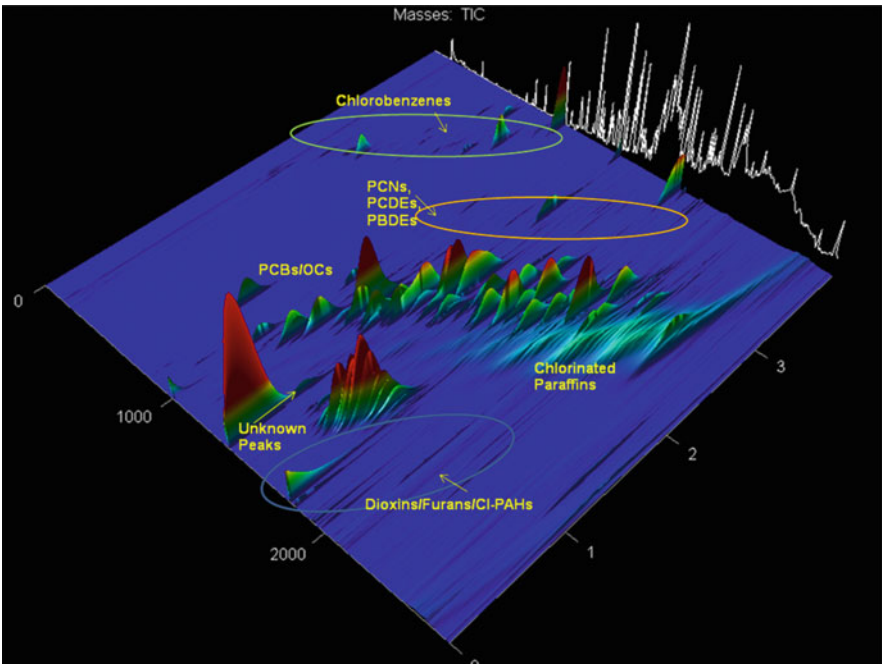

Fig. 5 Three-dimensional GCxGC-ECD plot of a sediment sample, private communication from Alina Muscalu using the method from [116]

selective detectors to be used [116, 117]. The significantly increased peak capacity of this technique enables multiple analyte groups like dioxin, PCBs, organochlorine (OC) pesticides, PCNs, and other organohalogen compounds to be analyzed in the same single run in many cases bypass the need for sample extract fractionation. Cryogenic zone compression modulation (CZC) has been used to enhance sensitivity of the analysis of dioxin in blood samples [118]. In this technique, a single 7 m, 0.1 mm id, and 0.1 μm d_f column is fed through a modulator. A long modulation period of 6–9 s is used to attempt to trap the entire peak into one modulation. An on-column detection limit of 300 attograms which is about an order of magnitude lower than conventional methods can be achieved.

5 Mass Spectrometric Detection

A number of different types of mass spectrometers are capable of analyzing dioxin. The magnetic sector high-resolution mass spectrometer (HRMS) was the first used in the early 1970s and is still the most sensitive and selective instrument for dioxin analysis [119–121]. It is considered the gold standard for dioxin analysis and is the only type of mass spectrometric detector allowed for a number of regulatory

analytical methods for litigation purposes. GC-HRMS is the most technically complex system and requires the most highly skill operators. There are a number of reviews comparing different mass spectrometric system [26, 27, 105, 122–130]. GC-HRMS must be operated in the selected ion monitoring (SIM) mode where only ions from the analytes are scanned. In this process, the magnet is set to a specific mass range, and the accelerating voltage is jumped to pass ions with mass-to-charge ratios only of the ions of interest, because the instrument only spends time directly on desired analytes and not scanning a range of masses that in most cases will not produce a signal, SIM can enhance sensitivity up to two orders of magnitude and is required in order to meet the very low detection limits needed for dioxin analysis. HRMS instruments are operated at a resolution of 10,000. Regulatory methods require the resolution to be confirmed every 12 h. This was a challenge for instruments in the late 1970s and early 1980s. Modern instruments can now hold 10,000 resolutions for days. HRMS instruments require a mass calibrant like perfluorokerosene (PFK) to ensure mass accuracy. PFK is used to tune the mass spectrometer and calibrate the mass scale. It is then infused into the mass spectrometer during the analytical run as a lock mass to ensure there is no mass drift. The lock mass trace is monitored to ensure results are not biased by matrix suppression.

Tandem mass spectrometry using triple quadrupoles [131, 132] has been used for the analysis since the mid-1980s. Recently, tandem quadrupole mass spectrometers have been used and approved in Europe specifically for food testing and food safety purposes [133–135]. Hybrid mass spectrometers (HRMS/quadrupole) [136], ion trap mass spectrometers [137–139], and the orbitrap [140, 141] have also been used for tandem mass spectrometry analysis of dioxin. Hybrid instruments are expensive and even more difficult to set up and operate than HRMS systems and are no longer routinely available commercially. Ion traps have been used mainly for screening purposes because being a trapping-type mass analyzer, ion suppression from coextractable compounds can result in loss of sensitivity or bias. The orbitrap can meet method requirements of regulatory dioxin methods and may also become a replacement for magnetic sector instruments in the future.

Tandem mass spectrometry uses multiple reaction monitoring (MRM) where the molecular ion is selected by the first mass analyzer and is then passed through a collision gas (typically nitrogen or argon) in a second quadruple set to pass all ions. Characteristic fragment ions are then selected by the third quadruple (mass analyzer). Dioxins lose COCl (63 Da) in MRM transitions and are the only group of compounds that lose this specific neutral fragment producing clean mass chromatograms with very few interferences [132].

Most dioxin methods also determine results for the dioxin-like PCBs. Polychlorinated biphenyls do not have a unique MRM fragmentation reaction like the loss of COCl from PCDD/PCDFs. The major fragment reaction for PCBs is the loss of Cl_2. Most polychlorinated compounds lose Cl_2, and therefore MRM chromatograms for PCBs can exhibit a significant number of interferences.

The sensitivity of newer triple quadrupole mass spectrometers can approach that of HRMS instruments using MRM. Applying additional mass selection stages to the

analytes of interest reduces their transmission through the instrument resulting in a reduced signal. The additional mass filtering however reduces the chemical noise at a greater rate than the loss in analyte signal, therefore resulting in net increase in the signal-to-noise ratio. GC-MS/MS has been accepted in a number of dioxin methods as an alternative to HRMS including the analysis of food and feed [23, 24, 134].

A number of adjustments that can be used to increase sensitivity include using larger sample sizes or sample volumes (or PTV/LVI injection) and/or microbore columns. A number of applications using large volume injection (LVI) or programmed temperature vaporization (PTV) have been reported. Programmed temperature vaporization enables a greater portion of the sample extract to be injected into the mass spectrometer increasing sensitivity and allowing smaller sample sizes to be used [142–146]. It also enables minimal sample concentration. Bias resulting from analyte loss for semivolatile compounds like dioxin and PCBs is typically not significant, especially when isotope dilution mass spectrometry is used. The major advantage is time savings from not having to concentrate organic solvents.

5.1 Ionization Techniques

Electron ionization (EI) is the classical ionization method for dioxin. It is available in essentially all GC-MS instrument types. Electron ionization is normally carried out using 70 eV electrons which produce ions with a broad range of internal energies resulting in variety of different fragment ions [147]. When using SIM, the number of possible ions produced should be as few as possible. In order to minimize the degree of fragmentation, an electron energy of about 35 eV should be used. The reduced electron energy decreases the degree of fragmentation and enhances the molecular ion signal which correspondingly enhances sensitivity. The mass spectra of halogenated compounds exhibit multiple isotopic peaks for the molecular ions as well as their corresponding fragment ions. Sensitivity is reduced due to these isotopic ion clusters, because the signal is split over the isotopic ions. This is also an advantage as isotopic ratios are much more stable than fragmentation ratios, and this information can be used for identification and conformation of the analytes of interest. Specific abundance of molecular or fragment ions is monitored and compared to acceptance criteria. This can be important in identifying potential interfering compounds that may be present. If interfering compounds are routinely presents, analysts can select other isotopic peaks which may be free of interferences for quantification.

Chemical ionization (CI) [148] is a soft ionization technique producing mass spectra with little fragmentation where the molecular anion is typically the base peak [149, 150]. Electron-capture negative-ion chemical ionization (ECNI) is a form of NCI using a reagent gas like methane that is to adsorb energy from electrons so they can be more easily attached to analytes of interest. The sensitivity of most polyhalogenated compounds like PCDD/PCDFs increases with increasing

degree of chlorination in ECNI, and for most congeners, the sensitivity in NCI can be much greater than with EI. However, for 2,3,7,8-T_4CDD, as for a number of organochlorine pesticides, the Cl^- anion [151, 152] is the base peak resulting in a 2,3,7,8-T_4CDD molecular anion with much lower intensity in NCI than that of the molecular ion in EI. Due to the presence of many other chlorinated compounds in most sample extracts, the Cl^- anion is common, and therefore monitoring Cl^- is not very selective. Chemical ionization using O_2 as a reagent gas has also been investigated [149, 153, 154]. It can be used to distinguish different congeners, but is much more difficult to use than EI and not sensitive enough to be used for ultra-trace analytical purposes with regular chemical ionization vacuum sources.

Atmospheric pressure ionization is a new technique that has been available for a few years. It ionizes target molecules by charge exchange from the nitrogen present in the source which has been ionized from a corona discharge [155, 156]. The advantage of atmospheric pressure ionization is that it can be matched with other atmospheric liquid chromatographic sources enabling instruments to be used as a more flexible combined GC and LC instrument. There are only a few reports of atmospheric pressure gas chromatography (APGC) for dioxin using tandem quadrupole mass spectrometry [156, 157]. APGC-MSMS is an excellent combination where the ion sources are based on a modified LC source. Both APGC source and the tandem mass analyzer are simple to maintain and operate, resulting in a rugged, sensitive instrument with minimal downtime. Figure 6 shows the sensitivity of this new ionization technique.

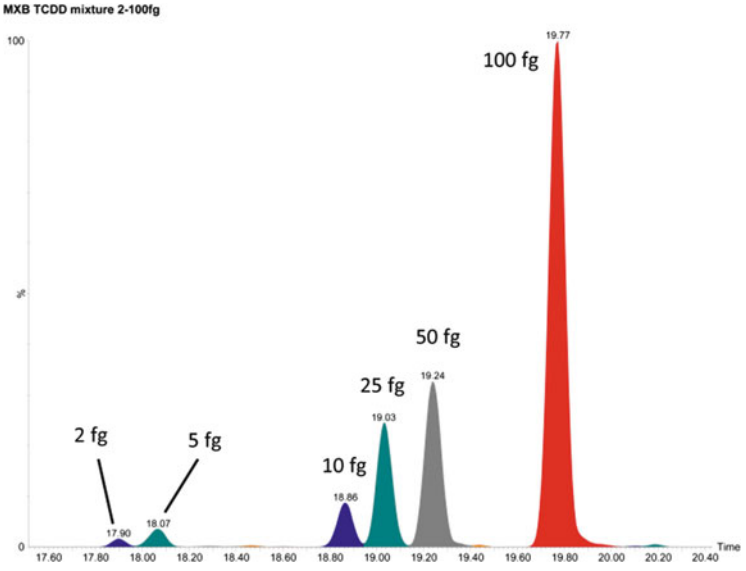

Fig. 6 Chromatogram demonstrating the sensitivity of the APGC-MS/MS. Congener identifications are, from *left* to *right*, 1,3,6,8-, 1,3,7,9-, 1,3,7,8-, 1,4,7,8-, 1,2,3,4-, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Reproduced with permission from [158]

5.2 Calibration and Quantification

Quantification of dioxin and dioxin-like compounds is a challenge because of the large number of congeners and the many cleanup stages required. In order to obtain good-quality data, isotope dilution with ^{13}C-substituted PCDD/PCDF internal standards (surrogates) for quantification of the 2,3,7,8-containing congeners is used. In addition to using mass-labeled internal standards for quantification, they are also used as GC retention time markers to ensure that the correct native toxic isomer is detected. Isotope dilution is the most accurate form of quantification [159, 160]. Adding labeled surrogates at the beginning of the analytical procedure enables results to be corrected for any losses during the multistage sample preparation procedure, sample concentration steps, volumetric transfers, or instrumental variability. Additional labeled internal standards can also be added at different stages. Cleanup standards are added after extraction to evaluate extraction and cleanup efficiency. Injection or syringe standards are added before injection to correct for injection variability and instrumental drift.

A calibration curve with five or more concentrations is typically used. The calibration standards typically contain all 17 native 2,3,7,8-substituted congeners and 16-labeled 2,3,7,8-substituted congeners. Labeled OCDF is not included because the higher mass isotopes of labeled OCDF can interfere with native OCDD. Concentration of the lowest-level calibration standard (CS1) for 2,3,7,8-TCDD is typically 0.5 pg/μL. Concentrations increase by factors of 4 or 5, to a concentration of 200 pg/μL in CS5, the highest-level calibration standard. Levels of the penta to hepta congeners are typically a factor five higher than the tetra concentrations and OCDF and OCDD which are typically 10 times higher. Examples of calibration standard sets can be seen in any of the methods listed in Table 2. A relative response factor (RRF) is calculated between the native analyte and labeled surrogate for each 2,3,7,8 substituted congener except 1,2,3,7,8,9-HxCDD whose labeled standard is used as an injection standard and OCDF. The average RRFs of the other two HxCDD congeners are used to quantify 1,2,3,7,8,9-HxCDD, and the response factor of OCDD is used for OCDF. Relative response factors are used to correct for any difference in response or concentration of the labeled standards relative to the native compounds in the calibration standards and should be close to 1. Non-2,3,7,8-substituted congeners are quantified using the average RRFs determined to their specific congener group.

In most methods, an average response factor over the calibration range is used instead of a response factor from linear regression using a classical calibration curve. Response factors (RFs) for the labeled surrogate standards are determined using internal standard calibration by the ^{12}C-labeled injection standards (1,2,3,4-T_4CDD and 1,2,3,7,8,9-HxCDD). Concentrations of the surrogates are determined and reported as percent recoveries which are used for quality control purposes.

5.3 Detection Limits and Low-Level Quantification

Dioxin is considered the most toxic organic chemical, and as such, analytical methods must be able to report results at sub-picogram (10^{-12}g) levels and well into the femtogram (10^{-15}g) level for food analysis. At these low levels, background levels can easily contribute to contamination resulting in positive values in the laboratory blanks which often is the limiting factor and the determination sample detection limits [161]. This can usually be controlled for dioxins and furans in most cases; however, contamination from PCBs poses a larger problem. The use of PCBs in many building and electrical equipment remains and circulates in the building and laboratory area. Dust control, air filtration, and good laboratory practices can minimize background contamination. If levels are constant, background subtraction can be used [162].

In the analytical scheme, there are a number of detection limits that need to be considered. Instrumental detection limits can be used to determine sample sizes and sample extract volumes needed to obtain required detection limits. Average recoveries and background blank levels also need to be considered [163]. Method detection limits (MDLs) are a statistical determination of detection limits using a set ($n \geq 7$) of low-level spiked samples (spiked at 5–10 times the instrument detection limit) that are taken through the entire method [159, 160, 164] The MDLs are determined by multiplying the standard deviation (σ) of the mean concentration of the sample set by the student's T value. In this method, the value of the MDL of a compound can be elevated if there is contribution from background contamination. Because of the low levels required, many labs calculate the actual detection limit (DL) for each parameter in every sample [165]. In this method, the signal-to-noise ratio of five to one (peak to peak) or three to one (average to peak) is used to estimate the area of a peak that would have been present at the retention time of the target analyte. The estimated area value is in the concentration calculation. The advantage of this method is that it is an actual determination of the detection limit for the target analyte in each sample being analyzed. This method is not always used because it is very time-consuming. The limit of quantification (LOQ) is typically about three times the method detection limit value and is the level where the analyte signal is repeatably identifiable, discrete, and reproducible with a precision of about 20% and an accuracy of ±20% [80, 159, 165]. The LOQ is important parameter for regulatory and litigation situations. In such cases, sample size and concentrations factors should be used such that results are well above LOQ values. In Ontario Canada, LOQ values must be greater than 10% of the regulatory limit if technologically possible.

Any compounds found in laboratory blanks can elevate detection limits and/or result in biased high values. Correction for background contamination can be carried out if blank levels are relatively constant (RSD <30%). This is often the case for low-level sample analysis like biological or human samples [80, 165]. Background contamination in blanks and reduced analyte recovery (as indicated by low recovery of labeled surrogates) can also elevate detection limits. Adsorption to

surfaces or cleanup packing materials is a major source for reduced analyte recovery. Bias can be magnified in low recovery situations (<40%). The correction of values for surrogate standard recovery also magnifies any value by the same amount as the recovery correction including the contribution for background contamination. The main advantage of using isotope dilution methods is that labeled analogs are often spiked into samples at levels significantly greater than those in the sample which can reduce losses due to adsorption effects. For other environmental samples like sediments, sludge, ash, or hazardous waste where levels in samples can vary up to eight orders of magnitude, blank levels can fluctuate significantly. In this case, determining accurate values for blank correction can be very difficult, and this may result in significantly elevated uncertainty values (i.e., >100%).

Other sources of bias include polychlorinated diphenyl ether (PCDE) fragment ions for PCDFs. PCDEs fragment by loss of Cl_2 to form PCDFs in the mass spectrometer ion source. PCDE traces are typically monitored to ensure there is now column break through during carbon column sample preparation.

6 Screening and Alternate Methods

Dioxin analysis is the most complex, time-consuming, and labor-intensive analytical method. Screening methods can be used to increase analytical capacity and reduce costs. They can be very valuable for screening samples like food and feed where dioxins are not expected or contaminate sites where they are used to provide quick results to enhance site cleanup procedures [166, 167].

The high cost of analysis for dioxin has resulted in the development of a number of alternate less expensive and quicker analytical methods. Bioassays [168–173], immunoassay [174–177], and aryl hydrocarbon receptor-based polymerase chain reaction (PCR) assays [178] have been developed. For bioassays to produce accurate results and compare very well with the classical HRMS methods [179, 180], a rigorous dioxin-type cleanup sample cleanup procedures must be used.

Screening approaches include instrumental methods monitoring a reduced number of congeners, reduced resolution methods, and a variety of bioanalytical methods. Schrock et al. [181] have compared the analysis of dioxins in soils using a modified USEPA-1613b GC-HRMS method. Samples were extracted using PLE, extracts outside the calibration range were not diluted and reanalyzed, and no secondary column confirmation (DB-225) was used. The majority of results compared within 10–20% with the classical USEPA-1613b method [30]. In most cases, contribution of compounds like 2,3,7,8-T_4CDF after multiplication by their respective TEF is significantly less than the uncertainty of the method, and their contribution to the TEQ is minimal indicating that confirmation by a secondary column may not be required in most cases.

Bioassay and immunoassay methods do not require complex expensive instrumentation for analyte detection enabling any laboratory with basic equipment to

analyze samples for dioxin. However, it is still critical that proper sample preparation procedures are used. The inability to use labeled internal standards creates challenges with respect to analyte loss during sample preparation, and cross-reactivity from compounds with similar structures can result in biased results. Dindal and Billets [182] compared a number of bioassay/immunoassay methods used in an intercalibration/method validation study. Participating laboratories were typically biased high for dioxin/furan and PCB and less precise when compared to HRMS. Detection limits ranged from approaching those of HRMS to more than two orders of magnitude higher. The number of false positives ranged from 6 to 10%, and the number of false negatives ranged from 0 to 8%. Analytical costs were significantly lower ranging from 5 to 50% of HRMS, and analysis times were less than 10% that of HRMS. Nording et al. [183] compared two bioassays and one immunoassay method with HRMS. The bioassays tended to overestimate results due to contributions from cross-reactivity of other dioxin-like compounds in the sample. If an extensive cleanup was used, no statistical difference between the bioassay and HRMS results was observed.

7 Quality Assurance/Quality Control/Accreditation

The analysis of dioxins and related compounds requires an extensive QA/QC program to ensure that analytical procedures are developed and validated by methods that can produce accurate, precise, and ultra-trace results in order to meet required data quality objectives [184–186].

There are numerous quality control limits and ranges that must be met for each set of samples. These include limits such as:

1. An acceptable range for calibration and continuing calibration checks. The relative standard deviation in the value of RRFs determined as the mean RRF across the calibration curve range should be less than 20%. This is typically the largest contribution to the uncertainty of the analyte. The value for continuing calibration acceptance is also $\pm$ 20% of the mean RRF value for the specific congener.
2. An acceptable range for recoveries of surrogates from 25 to 150% in most regulatory methods [30]. Recoveries of less than 10% to over 200% can be observed resulting from suppression or enhancement in signal for extracts that contain significant amounts of coextractable matrix materials. If this occurs at the retention time of the injection standards and/or labeled internal standards, significant bias in the result for the recovery of the associated internal standards can occur, and analysts should check the lock mass trace for any dips or disturbances. Changes of more than 10% in the level of the lock mass can result in bias, and further cleanup of the sample extract or dilution and reanalysis should be done.

3. Elution of target analytes within the proper elution window as defined by the "Window defining standard" which contains the first and last of every dioxin and furan. This standard is analyzed daily or after any GC column maintenance.
4. Ability to separate the 1237/1238 coeluting pair and 2378-TCDD at a valley of 25% or lower on a 5% phenyl-methyl column. This is the critical pair for 2378-TCDD separation and column performance.
5. Resolution check to ensure the instrument is operating at a resolving power of 10,000 or greater. This check is required least every 12 h for most regulatory methods.
6. GC peak shape of the native dioxin must be Gaussian and eluting within 2 s of its corresponding ^{13}C-labeled standard.
7. The isotopic ratios must be within 15% of their theoretical value. Values and ranges are listed in most regulatory methods.

Text box 1 summarizes the key considerations in the development of an analytical scheme for the analysis of dioxin and other POPs. Due to the ultra-trace nature of dioxin analysis, extra care must be taken to ensure that contamination for background levels and carryover does not result in significant analytical bias. Isotope dilution mass spectrometric procedures, IDMS, enable the correction for losses in the extraction and cleanup stages [187] as well as correction for any variability in the analysis stage. The $^{13}C_{12}$-labeled standards act as time markers for the native analyte being determined to ensure that the 2,3,7,8-labeled congeners are correctly identified even if there is any shift in retention times. The recovery of the surrogate standards effectively turns every sample into its own spiked matrix sample providing important quality control information. Different surrogates can be added at different stages of the sample preparation procedure and can be used to investigate any problems in the analytical procedure. ISO/IEC 17025 lists a number of critical quality control checks required to produce quality data. Key components of a quality assurance program as well as some of these critical quality control checks are listed in Text Box 2. One important consideration is that the analytical methods that are used are validated to ensure the methods are rugged and produces data that are accurate and precise.

Participation in interlaboratory studies and the use of reference materials can be used to assess accuracy and precision [188, 189] Certified reference materials (CRMs) are important tools for performance evaluation to ensure the laboratory is reporting accurate and precise results. A number of different CRMs for Dioxin have been developed in the past few years [190–193].

Van Bavel and Abad [194] reported a significant enhancement of the quality in dioxin data over the previous 15 years. They concluded that through a series of interlaboratory studies, issues with sample preparation, chromatographic separation and instrumental detection had been identified and corrected and that limits for confirming that methods and procedures are in control and "fit-for-purpose" were $\leq$10% RSD for standards, $\leq$15% RSD for samples extracts and $\leq$20% RSD for soil/sediment or ash samples. The uncertainties for dioxin methods are typically in the order of $\pm$20% per congener [123] which agrees very well with the limits

reported by Van Bavel and Abad [194]. The uncertainty of the TEQ tends to be lower (about 10%) than that of the individual congeners because it is the sum of the uncertainties of each of the individual congeners [123].

However, depending on how TEQs are calculated, there can be significant bias or error when comparing TEQs. It is important to identify the specific TEF scheme used and how the TEQs were calculated. TEQs can be calculated a number of different ways using different values for detection limits, e.g., ND = 0, ND = 1/2ND, ND = $ND^{0.5}$ or ND = ND. At lower levels, there can be a significant difference in TEQ values depending on which detection limits (value for ND) used varying up to 50% [162, 194].

Laboratory accreditation is an important requirement for routine analytical laboratories. Accreditation to ISO/IEC 17025 – General requirements for the competence of testing and calibration laboratories [195], is the standard to which labs are deemed technically competent.

Text Box 1: Key Considerations in an Analytical Scheme for POPs Analysis

- Ensure all labware, reagents and analytical instruments are free of contamination and interferences before beginning analysis
- Detect analytes at levels to meet MDLs – e.g., to meet sub-picogram detection limits for dioxin, every piece of labware should be prechecked (<500 fg) or labware segregated for high and low samples
- Determination of a representative sub-sample including gravimetric or volumetric determination for analysis. May require adjusting sample size or replicate analyses
- Addition of all internal and surrogate standards to the sample such that they will behave as the natural analytes in the sample during the analysis minimizing potential bias
- Quantitative extraction of analytes from matrix
- Extract may contain significant amounts of coextractable organic material including: Dioxins, Furans, PCBs PCNs, PCDEs, polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCs), brominated flame retardants (BFRs), and many other organic compounds including lipids, humic material and sulfur
- Cleaning of extracts to remove interfering coextractables to the degree where DQOs and QC limits can be met for the required analysis
- Not all coextractable compounds need to be removed, but they should not affect the separation or detection systems with respect to the analytes of interest
- Separation of target analytes from non-target or nontoxic isomers, congeners or interfering compounds (e.g., GC-HRMS for dioxin)
  - There are many congeners per analyte group

(continued)

Dioxins/furans: 210; PCBs: 209, PCNs 75

– Separate and accurately quantify all toxic congeners

Dioxins/furans: 17, PCBs: 12, PCNs: 8–10

- Ensure method and instrument selectivity and sensitivity meet DQOs and QC limits
- Accredited laboratory (to ISO 17025), Quality System, trained/experienced analysts, proper instrument and analytical procedures, validated and documented methods and SOPs, control charting, non-conformance and root cause determination
- Ensure quantitative accuracy
  - Calibration, blanks, spiked samples, CRMs, ILS, Performance Evaluation, surrogates and internal standards, standard validation.
- Other considerations:
  - Toxicity of compounds can range up to six orders of magnitude. For example, T_4CDD toxicity can range from: NOEL = 3 g/kg to $LD_{50} = 1$ μg/kg – must identify correct isomers using proper GC columns and conditions.
  - Range of concentrations – fg/g (10^{-15} g/g) to % levels – potential lab contamination and instrument issues from carryover.
  - Range of sample types and complexities – biota, air, water, soil, hazardous waste which have different matrix dependent and method requirement issues. Use method fit for purpose that has been validated for specific matrix being analyzed.
  - Are the patterns of congeners representative of samples being analyzed, e.g., does the pattern match an Aroclor mixture (PCB) or does an ash sample have a combustion source pattern or does a biota sample have only the toxic congeners present (dioxin)?

Text Box 2: Key Steps for Quality Assurance and Quality Control

A Comprehensive Quality Assurance Program Includes:

- A Quality Management System that includes a Quality Manager, Quality Management group, quality manual and procedures manuals (for non analytical procedures)
- Detailed written analytical methods and standard operating procedures (SOPs)

(continued)

- Document management system to ensure controlled documents and approved procedures are used
- Facilities management – proper working conditions (temperature control, light and power) and supply of water, air (fume hoods), and IT (networks, data system and storage)
- Human Resource management to ensure trained and proficient analysts
- Health and Safety Committee and protocols to ensure safe working conditions
- Sample management – proper reception, logging, storage and distribution to ensure samples do not spoil
- Procurement of equipment and supplies – proper instruments, labware, data systems, software
- Preventative action (maintenance) – procedures to ensure failures are minimized, e.g., change pump oils on regular schedules
- Corrective action – system to review failures or non-conformance and determine root cause to ensure problem will not reoccur
- Standardization and calibration of instruments, volumetric labware, balances, etc
- Research and development, continual improvement
- Performance evaluation/review: intercalibration, round robins to determine accuracy and ruggedness of method
- Accreditation – external review to ensure lab is following proper procedures, e.g., ISO 17025
- Control charting – data trend analysis to ensure data quality
- Determination of uncertainty

Critical Quality Control Checks Include:

- Control standard – to check calibration and accuracy (alternate source standard) – determines accuracy of calibration
- Low level standard – confirm sensitivity of instrument and detection limits can be met
- Column performance check – should contain critical pair showing analytes are resolved chromatographically, e.g., 1237/1238-TCDD and 2,3,7,8-TCDD
- Blanks: laboratory (procedure), field, reagent, instrument blanks – to determine levels of contamination
- Control sample: spiked samples/reference materials – determines overall accuracy of method and data set
- Internal standards/surrogates – determines recovery and accuracy of analytes in samples
- Duplicate/replicate – determines precision

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8 Related Dioxin-Like Pops

There are a number of other compounds that interact with the Ah receptor and have dioxin-like toxicity. These include brominated and mixed halogenated dioxins and furans, polychlorinated naphthalenes and halogenated carbazoles [196–201]. Brominated dioxins/furans can be formed by thermal degradation of PBDEs and, bromo/chloro exchange can occur to form mixed bromo/chloro dioxins/furans [202]. At lower temperatures (between 250°C and 500°C). Rupp and Metzger [203] reported that bromo/chloro exchange occurs on the PBDE molecule prior to dehalogenation and ring closure.

Methods for most halogenated compounds use common extraction and cleanup and techniques [204, 205]. Polybrominated dioxins and furans [198, 206, 207] can be analyzed under similar conditions to those for chlorinated dioxins and furans, however shorter thin film columns should used and care must be taken to limit contamination from PBDEs and degradation from light and heat [207–211].

Mixed halogenated dioxins and furans are the most challenging type of dioxins to analyze. There are 1,436 mixed bromo/chloro dioxins congeners [212–214] and chloro/fluoro dioxins [215, 216] and only a few bromochloro standards are available commercially. Mixed halogenated dioxins and furans are at least as toxic as chlorinated dioxins [217]. Van den Berg et al. [16] reported that the ranges of relative potencies determined from various studies of chlorinated, brominated and mix halogenated dioxins, furans and dioxin-like PCBs overlapped and therefore, TEFs for the most recent chlorinated compounds should be used in TEQ calculations.

Levels of mixed halogenated dioxins are typically an order of magnitude or more lower than the chlorinated PCDD/Fs [218, 219]. The use of GC-MSMS where the loss of COCl and COBr is monitored significantly increases selectivity over HRMS methods [220]. The increased sensitivity of APGC has helped lower detection limits for analysis [156, 158, 221]. Multidimensional (GCxGC) chromatography has also been used [222].

9 Future Trends

There are significant challenges to enhance sensitivity, selectivity and speed for the analysis of Dioxin and dioxin-like compounds while reducing analytical costs. Dioxin sample preparation methods are evolving toward more automated or semi-automated extraction and sample preparation systems that analyze multiple analyte groups of POPs including polybrominated dioxins/furans, PBDEs, and PCNs. The current trend is toward lower detection limits as toxicity and risk is now better understood. Analytical instrument sensitivity is much lower in recent years and blank levels are the limiting factor in reducing detection limits in many cases. The current limitation is the availability of analytical instrumentation that can separate

and detect the many closely related compounds at levels low enough for protection of human health and the environment in a single analytical run. More sensitive multi-analyte group screening techniques that require reduced sample preparation as a result of the advancement of chromatographic techniques like multidimensional chromatography and fast scanning mass spectrometric detectors will greatly aid in the advancement for the analysis of Dioxin and related dioxin-like POPs. The development of full scanning analytical instrumentation with the sensitivity and selectivity of current HRMS instruments could be used to analyze target and non-target compounds a levels low enough to detect Dioxins and provide results for other known or unknown compounds new and emerging compounds.

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Optimization of GC-MS/MS for the Determination of Dioxins and PCBs in Feed and Food and Comparison of Results with GC-HRMS

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Abstract Until recently, the analysis of dioxins and dioxin-like PCBs in feed and food was generally performed by gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) due to its superior resolution and sensitivity compared to the then available gas chromatographic techniques with other detectors. However, in the past few years the technology of GC coupled to tandem mass spectrometry (GC-MS/MS) has made great progress and modern GC-MS/MS systems nowadays reach similar limits of detection as GC-HRMS systems. This paper describes the step-wise optimization of GC-MS/MS variables for the sensitive and selective measurement of dioxins and PCBs in feed and food to comply with the performance criteria set by the EU Commission for confirmatory methods. The developed method was shown to give linear response over the required concentration range and good repeatability. Analyses of samples from official feed and food control demonstrated that a surveillance of the legal limits for dioxins and PCBs in feed and food is easily achievable. The generated quantitative results for dioxins and PCBs are compared with those data obtained for the same samples by GC-HRMS and show good agreement. As the purchase and running costs for GC-MS/MS systems constitute only a fractional amount compared to GC-HRMS instruments, the application of GC-MS/MS represents a cost-effective alternative for GC-HRMS to reliably and unequivocally confirm low levels of dioxins and PCBs in feed and food.

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Contents

Abbreviations

AL	Action level
Dioxins	Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans
DL-PCBs	Dioxin-like PCBs
ECNI	Electron capture negative ionization
EI	Electron ionization
EU	European Union
EU-RL	European Union Reference Laboratory
eV	Electron volt
fg	Femtogram
g	Gram
GC	Gas chromatography
HRMS	High resolution mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
mg	Milligram
ML	Maximum level
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
μg	Microgram
msec	Millisecond
NDL-PCBs	Non-dioxin-like PCBs
ng	Nanogram
NRL	National Reference Laboratory
PCBs	Polychlorinated biphenyls

PCDDs	Polychlorinated dibenzo-*p*-dioxins
PCDFs	Polychlorinated dibenzofurans
pg	Picogram
TEQ	Toxicity equivalents
V	Volt
WHO	World Health Organization

1 Introduction

For the general population, the major pathway to human exposure with polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), together often termed "dioxins" as well as polychlorinated biphenyls (PCBs) is via food ingestion. On average, diet contributes around 80–90% to total dioxin exposure, of which food of animal origin generally accounts for 70–80% [1]. A comparable situation holds true for PCBs. In the past 15 years a number of incidents were discovered where adulterated feed resulted in a widespread pollution of the feed and food chain with dioxins and PCBs [2]. These cases were often induced by criminal or grossly negligent action of individuals and caused seizure and destruction of tens of thousands tons of feed and food and slaughter of thousands of food producing animals.

Due to numerous technical and administrative measures, the release of dioxins and PCBs was considerably reduced in the past two decades resulting in diminished levels in the environment and decreased human exposure through dietary intake. These beneficial effects were often foiled by the consumption of contaminated food as a result of the before mentioned incidences. From a health point of view, this is of concern, especially when taking into account that a substantial part not only of the European population is still exposed to concentrations in the range of or even above the tolerable intake of dioxins and dioxin-like compounds [2]. As a consequence and a pro-active approach, the European Commission has, e.g., set maximum levels (MLs) for dioxins and PCBs in a number of feed and food commodities. While initially the MLs only referred to dioxins, they were later expanded to the sum of dioxins and dioxin-like PCBs (DL-PCBs) and, in addition to the sum of the six non-dioxin-like PCB (NDL-PCBs) congeners 28 + 52 + 101 + 138 + 153 + 180 [3, 4].

In addition to maximum levels, the EU Commission has also set action levels (ALs) separately for dioxins and DL-PCBs, respectively, in feed and food. The ALs which can be considered as an early warning tool [4, 5] to identify concentrations that are clearly above environmental background contamination, generally amount to approximately 2/3 of the MLs. If the ALs are exceeded, the respective product can still be marketed; however, the competent authorities should initiate investigations to identify the source of contamination and take measures to reduce or eliminate the source of contamination.

The major dioxin and PCB contamination cases in feed and food illustrated the need for fast and high throughput methods to identify and confirm non-compliant

samples and to trace back the contamination sources. Until recently, legislation in the European Union required the confirmation of suspect and positive dioxin and DL-PCB findings in feed and food by isotope dilution gas chromatography coupled to high resolution mass spectrometry (GC-HRMS). In the USA, the application of GC-HRMS for dioxin analysis in food is stipulated by the US-EPA Method 1613B. As the purchase, operation, and maintenance of GC-HRMS systems is costly and requires highly skilled experienced personnel, attempts were made since years to develop alternative analytical techniques that are less costly, but sensitive and robust enough to confirm dioxins and PCBs at the level of interest. Earlier efforts using GC/Iontrap MS and GC/low resolution MS occasionally showed promising results but finally failed as an alternative confirmation method because of limited long-term sensitivity in every day analysis and/or extensive needs for time-consuming clean-up steps to avoid an overlap with co-extracted disturbing compounds. Since some five years, the GC-MS/MS technology, in particular, due to enhanced ion source optics has improved considerably. Modern GC-MS/MS systems nowadays allow a reliable and reproducible determination of dioxins and PCBs down to low fg/µl levels in feed and food [6–18]. Following initial results that illustrated the potential of GC-MS/MS as an alternative confirmation tool, a core working group was formed within the European dioxin network consisting of the European Union Reference Laboratory (EU-RL), National Reference Laboratories (NRL) of the EU Member States for dioxins and PCBs in feed and food and several external scientific experts. The aim was to evaluate the capabilities of the GC-MS/MS technique and to elaborate analytical performance criteria that have to be fulfilled as a confirmatory method for dioxins and PCBs in feed and food. This latter practice follows the principle of the "criteria approach" which is generally applied in Europe for the determination of contaminants in food. Rather than prescribing fixed reference methods that have to be applied, the EU Commission lays down strict analytical performance requirements for sampling and analysis that have to be fulfilled. As long as these performance criteria are fulfilled and the method was shown to be fit for purpose, the analysts can apply their method of choice.

To evaluate the capabilities of the GC-MS/MS technique, several proficiency tests were conducted by the EU-RL with various food and feed commodities containing dioxins and PCBs at different concentrations. The overall performance of the results obtained by GC-MS/MS was satisfactory and equivalent to results generated with GC-HRMS. In 2014, this led finally to the revision of the two European legislative acts that lay down the analytical performance criteria for the confirmatory analysis of dioxins and PCBs in feed and food. The revised Commission Regulations (EU) No 589/2014 and No 709/2014 now consider the application of GC-MS/MS as an equivalent confirmatory method as GC-HRMS [19, 20].

The objective of this study was to develop a GC-MS/MS method for the determination of dioxins and PCBs in feed and food that complies with the performance requirements laid down in European legislation for confirmatory methods. The method should be sensitive and selective enough to reliably and

unequivocally identify and confirm suspect and non-compliant samples analyzed in the framework of official feed and food control.

2 Influence of MS/MS Parameters on Achievable Analytical Sensitivity

The following sections describe the step-wise optimization of GC-MS/MS variables for the sensitive and selective measurement of dioxins and PCBs in feed and food at the European legal limits. Most of the work was performed on an Agilent 7890/7000B GC-MS/MS system.

2.1 Dioxins

Generally GC-HRMS methods for dioxin analysis, even in electron ionization mode (EI), run at an ionization energy of around 30–40 eV. However, this energy is too low and does not result in measurable signals when applied to GC-MS/MS. As indicated in Fig. 1, which shows the optimization of the ionization energy for a number of native and ^{13}C-labeled dioxin congeners, considerable signals for the analytes were only obtained from 50 eV onwards. The measurements were performed in the MS mode separately for the ranges 45–70 eV and 70–100 eV, respectively. The peak areas represent the precursor ion (M^+). As usual in GC–low resolution MS analyses, the ionization energy is increasing with increasing voltage, has its optimum around 70 eV, and decreases slightly at higher energies. Therefore, for all dioxin congeners an ionization energy of 70 eV was chosen.

The loss of COCl from the molecular ion $[M\text{-}COCl]^+$ constitutes the most prominent MS/MS transition for dioxins in EI mode. In order to identify the best conditions for a sensitive determination, for each congener the collision energy was increased in a first iteration between 20 and 50 Volt in steps of 5 Volt each. In a following experiment, the optimum collision energy was determined around the maximum identified in the first iteration by changing the collision energy around this maximum in 2 V steps. This is exemplarily shown in Fig. 2. The choice of the collision energy has an impact on the limit of detection (LOD) that can be achieved for the various congeners. The optimal choice for the collision energy is congener-specific and thus was determined separately for each congener.

As several dioxins, in particular higher chlorinated congeners show a strong response in mass spectrometry using electron capture negative ionization (ECNI) mode [21], experiments were conducted to elaborate whether GC-MS/MS in ECNI mode could be used as a sensitive and selective tool for their determination at low concentrations. All experiments were performed with methane as a reagent gas. The results obtained are depicted in Fig. 3. It shows the different sensitivities for each

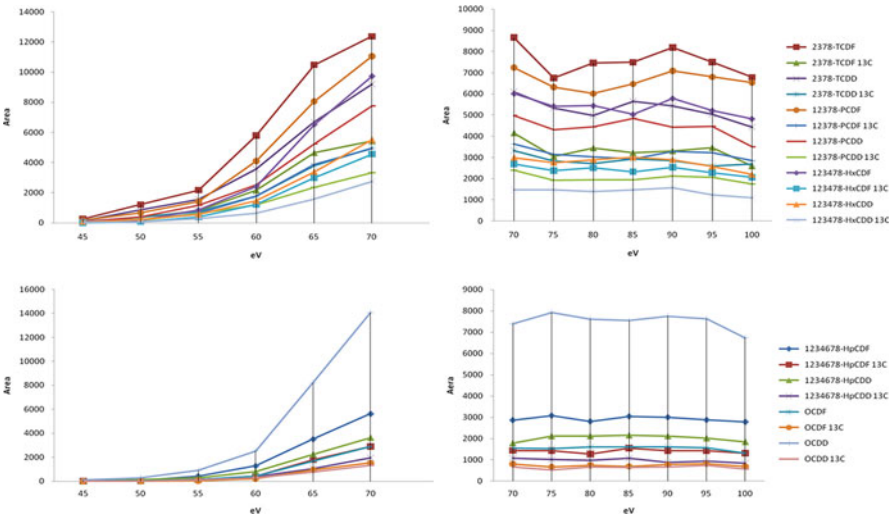

Fig. 1 Optimization of the ionization energy (eV) for native and ^{13}C-labeled dioxin congeners

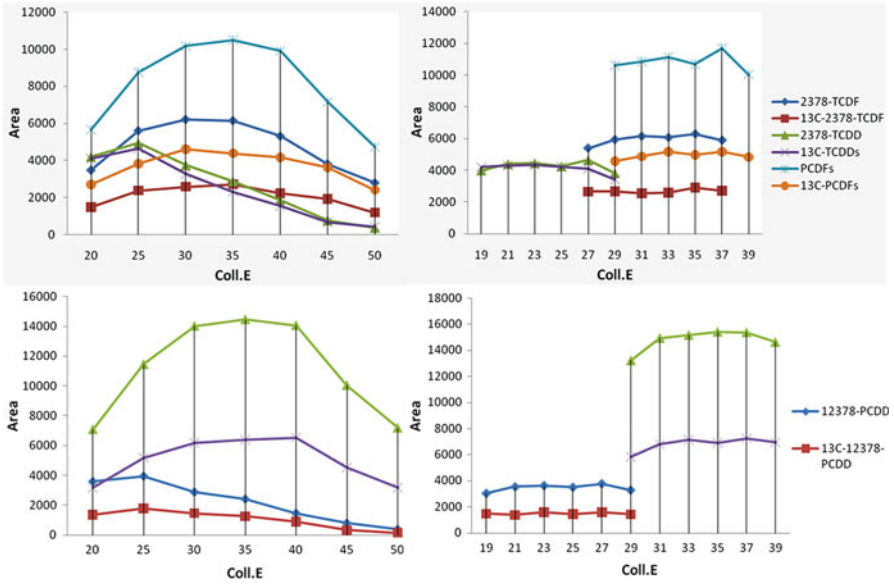

Fig. 2 Optimization of the collision energy for the product ions $[M\text{-}COCl]^+$ of selected native and ^{13}C-labeled dioxin congeners

congener in ECNI mode and the comparison with the GC-MS/MS analysis in EI for the respective compound. In each case, the optimal result for the various congeners analyzed in EI mode is standardized to 1. The determination of 2,3,7,8-TCDD with GC-MS/MS in ECNI mode at concentrations in the low pg/µl range is not feasible.

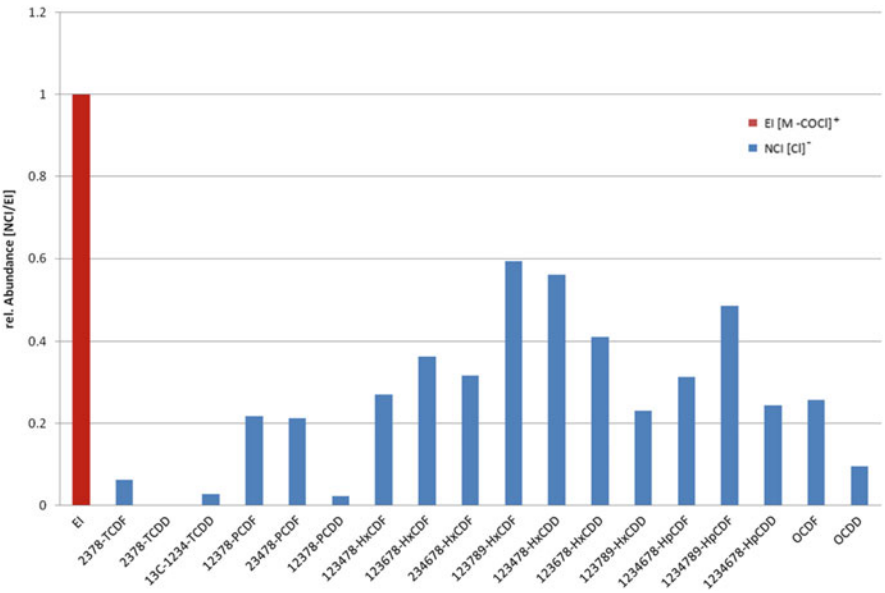

Fig. 3 Comparison of relative abundance for dioxin congeners analyzed with GC-MS/MS in EI and ECNI mode. The optimal result for the various congeners analyzed in EI mode is standardized to 1

Although the other congeners show somewhat better performance, none reaches the sensitivity that can be obtained by GC-MS/MS in EI mode. Thus, the application of EI is the method of choice for a sensitive determination of dioxins by GC-MS/MS.

Applying the optimal conditions for the determination of 2,3,7,8-TCDD by GC-MS/MS leads to limits of detection in the low fg/µl range (Fig. 4). The left chromatogram shows the MS/MS transition m/z $319.9 \rightarrow 256.9$ of a standard solution containing 2,3,7,8-TCDD at a concentration of 41 fg/µl (2 µl injected). In the right chromatogram the two transitions m/z $319.9 \rightarrow 256.9$ and $321.9 \rightarrow 258.9$ of that analysis are overlaid. The horizontal dotted lines indicate the maximum permitted deviation for the ratio of the relative intensities around the two transitions.

Recently, the sensitivity of the analysis was even further improved by introducing an enhanced ion source design of the mass spectrometer. This is demonstrated in Fig. 5 which shows the overlaid chromatograms of five injections of 10 fg 2,3,7,8-TCDD on column on an Agilent GC-7010 MS/MS system.

The linearity and sensitivity were measured for each of the 17 dioxin congeners with 2,3,7,8-chlorine substitution using a total of 17 ^{13}C-labeled internal standards over the concentration range of 0.05 pg/µl to 5 pg/µl with the exception of OCDD where the concentration range was 0.25–25 pg/µl (each 2 µl injected). Except for OCDD (0.9978), the correlation coefficients R^2 (linear fit) for all other congeners were >0.999, illustrating a very good linearity.

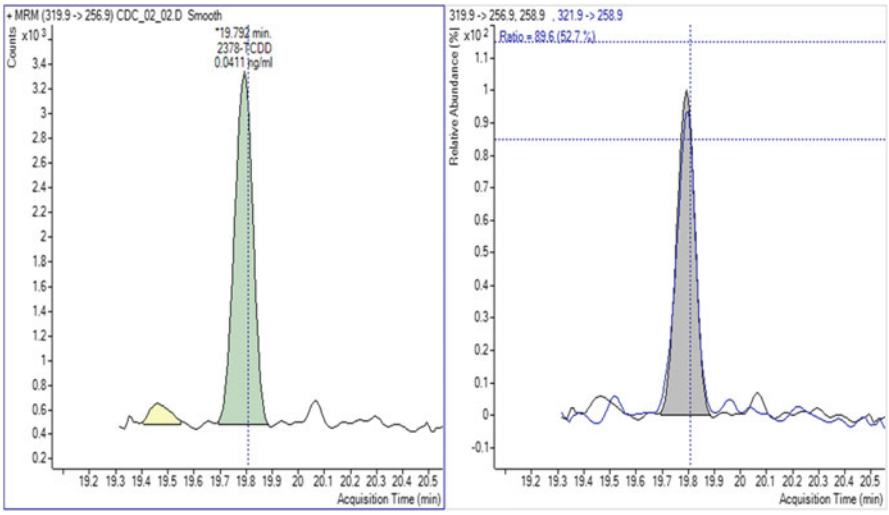

Fig. 4 GC-MS/MS analysis of 2,3,7,8-TCDD (41 fg/µl; 2 µl injected) Left chromatogram: MS/MS transition m/z 319.9 → 256.9; right chromatogram: overlay of the two transitions m/z 319.9 → 256.9 and 321.9 → 258.9 of that analysis with the ratio of the respective intensities

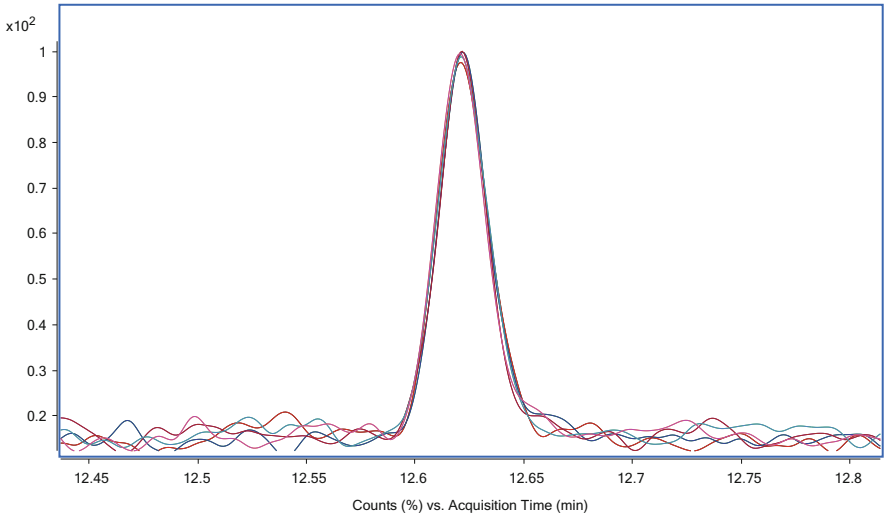

Fig. 5 Overlaid chromatograms of five injections of 10 fg 2,3,7,8-TCDD on column by GC-MS/MS

A problem in GC-MS/MS analysis is sometimes the very low noise level which generally hampers the calculation of a signal-to-noise ratio (S/N). This is especially important in dioxin analysis as all respective European maximum levels for dioxins in food and feed are upperbound concentrations. This implies that the numerical

value of the limit of quantification (LOQ) has to be used for the calculation of the Toxicity Equivalents (TEQ) in case a dioxin congener cannot be quantified in a sample. While by European legislation [19, 20] in GC-HRMS analysis, the LOQ has to be identified as the concentration of an analyte in the extract of a sample which produces an instrumental response at two different ions to be monitored with an S/N ratio of 3:1 for the less intensive raw data signal, a different approach has to be applied for GC-MS/MS analyses.

If for technical reasons the S/N calculation does not provide reliable results, as in the case of GC-MS/MS analyses, Commission Regulation (EU) No 589/2014 [19] stipulates that the LOQ of an individual congener may be identified as "*the lowest concentration point on a calibration curve that gives an acceptable (≤30%) and consistent (measured at least at the start and at the end of an analytical series of samples) deviation to the average relative response factor calculated for all points on the calibration curve in each series of samples The LOQ is calculated from the lowest concentration point taking into account the recovery of internal standards and sample intake*."

Due to the low noise level in GC-MS/MS analysis, smoothing of the mass spectrometer signals is problematic as it can "generate" peaks from noisy raw data potentially leading to false positive results. This is exemplarily illustrated in Fig. 6. The upper traces show the MRM transition m/z 441.7 → 378.8 for the native OCDF congener near the LOD (left: unsmoothed, right: smoothed) and the lower traces depict the MRM transition for the corresponding ^{13}C-labeled internal standard. While the unsmoothed signals do not reliably resemble a compound peak but seem to be generated by electrical or chemical noise, the smoothed peak looks like a compound and can easily pretend a substantial analyte concentration.

The optimized MS/MS conditions for the GC-MS/MS analysis of dioxins in feed and food are depicted in Table 1. Each native analyte and its corresponding

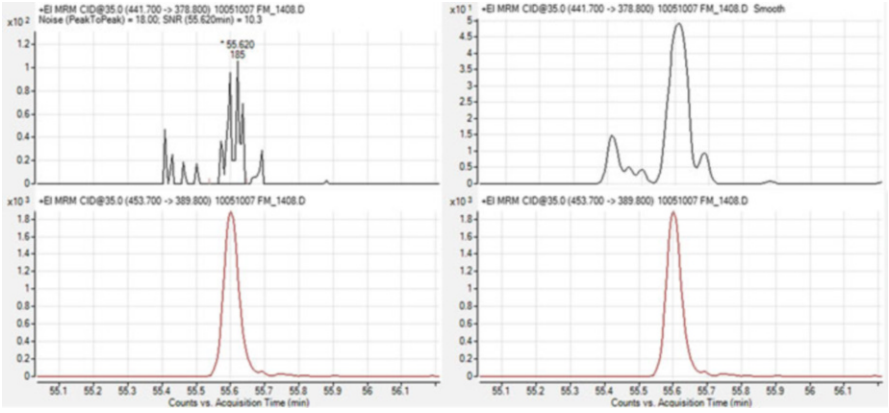

Fig. 6 "Generating peaks" through smoothing from noisy raw data; MRM transitions for native (upper chromatograms) and ^{13}C-labeled OCDF (lower chromatograms); left chromatograms unsmoothed; right chromatograms smoothed

Table 1 MS/MS settings for PCDDs, PCDFs, and ^{13}C-labeled internal standards

TS	Segment start time (min)	Analyte	RT (min)	Quantifier		CE (V)	Qualifier		CE (V)
				Precursor	Product		Precursor	Product	
1	25.0	^{13}C-2378-TCDF	35.43	315.9	251.9	33	317.9	253.9	33
		2378-TCDF	35.47	303.9	240.9	33	305.9	242.9	33
		^{13}C-1234-TCDD	35.77	331.9	267.9	24	333.9	269.9	24
		^{13}C-2378-TCDD	36.79	331.9	267.9	24	333.9	269.9	24
		2378-TCDD	36.80	319.9	256.9	24	321.9	258.9	24
2	40.0	^{13}C-12378-PeCDF	42.55	351.9	287.9	35	349.9	285.9	35
		12378-PeCDF	42.56	339.9	276.9	35	337.9	274.9	35
		^{13}C-23478-PeCDF	44.00	351.9	287.9	35	349.9	285.9	35
		23478-PeCDF	44.02	339.9	276.9	35	337.9	274.9	35
		^{13}C-12378-PeCDD	44.45	365.9	301.9	25	367.9	303.9	25
		12378-PeCDD	44.48	355.9	292.9	25	353.9	290.9	25
3	46.0	^{13}C-123478-HxCDF	48.04	385.8	321.9	35	387.8	323.9	35
		123478-HxCDF	48.06	373.8	310.9	35	375.8	312.9	35
		^{13}C-123678-HxCDF	48.21	385.8	321.9	35	387.8	323.9	35
		123678-HxCDF	48.22	373.8	310.9	35	375.8	312.9	35
		^{13}C-234678HxCDF	48.96	385.8	321.9	35	387.8	323.9	35
		234678-HxCDF	48.97	373.8	310.9	35	375.8	312.9	35
		^{13}C-123478-HxCDD	49.17	403.8	339.8	25	401.8	337.9	25
		123478-HxCDD	49.19	389.8	326.9	25	391.8	328.8	25
		^{13}C-123678-HxCDD	49.30	403.8	339.8	25	401.8	337.9	25
		123678-HxCDD	49.32	389.8	326.9	25	391.8	328.8	25
		^{13}C-123789HxCDD	49.63	403.8	339.8	25	401.8	337.9	25
		123789-HxCDD	49.65	389.8	326.9	25	391.8	328.8	25
		^{13}C-123789-HxCDF	50.04	385.8	321.9	35	387.8	323.9	35
		123789-HxCDF	50.06	373.8	310.9	35	375.8	312.9	35

4	51.0	^{13}C-1234678-HpCDF	51.84	419.8	355.8	36	421.8	357.8	36
		1234678-HpCDF	51.86	409.8	346.8	36	407.8	344.8	36
		^{13}C-1234678-HpCDD	53.11	437.8	373.8	25	435.8	371.8	25
		1234678-HpCDD	53.13	423.8	360.8	25	425.8	362.8	25
		^{13}C-1234789-HpCDF	53.69	419.8	355.8	36	421.8	357.8	36
		1234789-HpCDF	53.70	407.8	344.8	36	409.8	346.8	36
5	55.0	^{13}C-OCDD	56.23	469.7	405.8	26	471.7	407.8	26
		OCDD	56.24	457.7	394.8	26	459.7	396.8	26
		^{13}C-OCDF	56.41	453.7	389.8	35	455.7	391.8	35
		OCDF	56.42	441.7	378.8	35	443.7	380.8	35

^{13}C-labeled internal standard was measured using two precursor ions and two different product ions. The dwell times were each 75 milliseconds (msec) for the native and 25 msec for the labeled compounds. The MRM settings were split into five time segments, monitoring the tetra, penta, hexa, hepta, and octa PCDD and PCDF congeners, respectively. The MS transfer line was set to 300°C, the ion source to 280°C, and the quadrupoles to 150°C. Nitrogen at 1.5 ml/min and Helium at 2.25 ml/min were used as collision cell gases.

The GC column was a DB-5 ms UI 60 m × 0.25 mm, 0.25 μm with a 2.0 m × 0.25 mm uncoated Siltek deactivated fused silica pre-column. Helium was used as carrier gas at a constant flow of 1.0 ml/min. The oven temperature program was 130°C (2 min), 10°C/min to 200°C (16 min), 5°C/min to 235°C (7 min), and 5°C/min to 350°C. The method was retention time locked using PCB 105 to a retention time of 34 min. An automated liquid sampler with the sampler tray cooled to 5°C was used to make 2 μl pulsed cold splitless injections.

2.2 PCBs

The optimal conditions and settings for the GC-MS/MS analysis of PCBs were elaborated in a similar approach as for the dioxins. For this experiment, the 12 dioxin-like PCBs (DL-PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) as well as the six so-called indicator non-dioxin-like PCBs (NDL-PCB 28, 52, 101, 138, 153, and 180) were grouped into homologue groups based on their chlorine content. Figure 7 illustrates the ion yield of different homologue groups in dependence of the EI ionization energy which was increased initially by steps of 10 eV between 50 and 100 eV (left) and consecutively by steps of 5 eV around the maximum ion yield (right). The maximum sensitivity is reached around 78 eV for all homologue groups. When lowering the ionization energy down to 50 eV, a considerable loss in intensity can be observed, in particular for penta- and hexa-chlorinated PCBs. The same holds true when increasing the ionization energy up to 100 eV. Thus, an electron energy of 78 eV was used in all subsequent experiments.

After optimization of the ionization energy, the impact of the collision energy on the intensity of the product ions for the different PCB homologue groups was examined. For these experiments, the product ion with the highest response $[M\text{-}2\ Cl]^+$ was measured. The results are shown in Fig. 8. Initially, the collision energy was increased in steps of 5 V between 10 and 50 V (left) and subsequently in steps of 2 V around the maximum intensity (right). A strong impact of already small changes of the collision energy can be observed especially for the lower chlorinated PCBs while the hepta-chlorinated PCBs are obviously less prone to changes of the collision energy. The optimal conditions were around 30 V and set for each congener individually.

Besides the product ion $[M\text{-}2\ Cl]^+$ also the intensities of the product ions $[M\text{-}Cl]^+$ and $[M\text{-}3\ Cl]^+$ were examined for a potential MS/MS analysis. Figure 9 illustrates the summarized results of this comparison. The strongest signals for all homologue

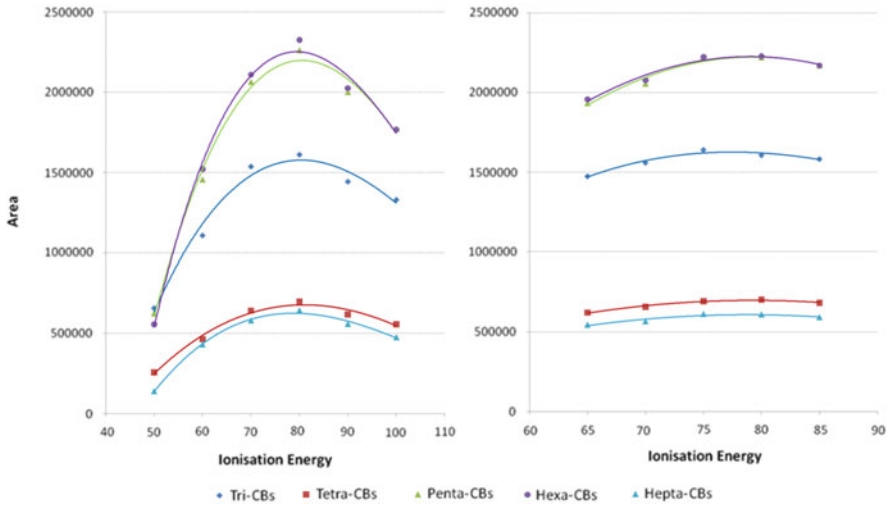

Fig. 7 Optimization of EI ionization energy for PCB homologue groups

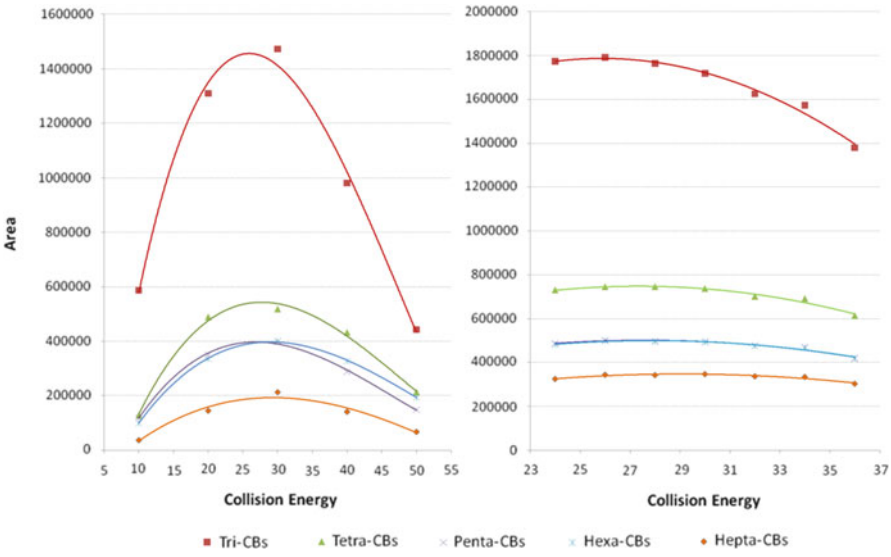

Fig. 8 Optimization of collision energy for PCB homologue groups

groups were observed for the product ion $[M\text{-}2\ Cl]^+$. Thus, the peak intensity for $[M\text{-}2\ Cl]^+$ for each homologue group was set to 1 and the relative intensities for the other two respective product ions at the optimal collision energy were calculated and plotted against this benchmark. Almost all intensities reached only 10–20% compared to the intensity of the $[M\text{-}2\ Cl]^+$ product ion. Only for tetrachlorinated

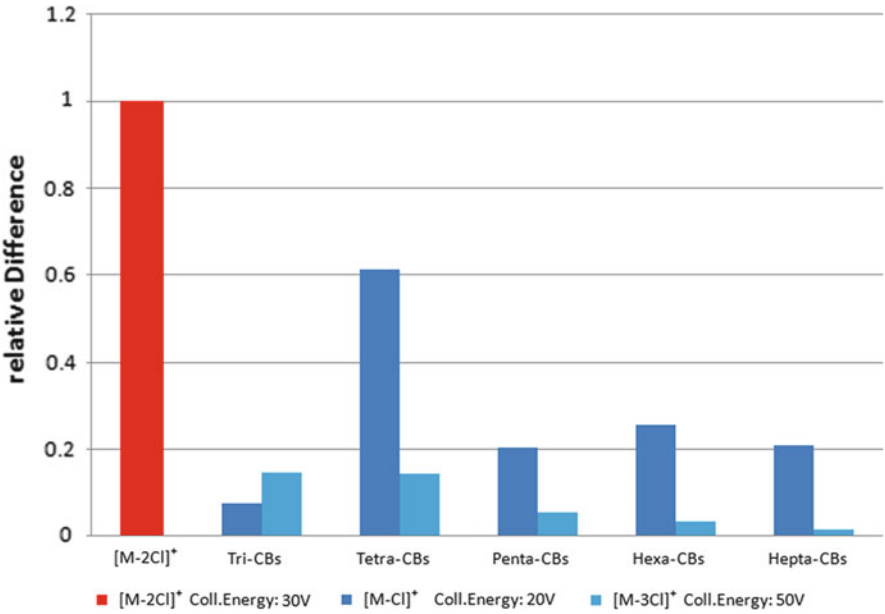

Fig. 9 Comparison of relative responses for different MS/MS product ions for PCB homologue groups. The peak intensity for [M-2 Cl]$^+$ for each homologue group was set to 1

biphenyls the intensity for the [M-Cl]$^+$ product ion amounted to approximately 60% of the most intensive MS/MS product ion.

Seven-point calibration curves were created for all 12 DL-PCB congeners over the concentration range of 0.05–10 pg/µl based on their corresponding ^{13}C-labeled internal standards (2.5 pg/µl each). The calibration curves for the 6 NDL-PCBs comprised the concentration range from 0.05 pg/µl to 50 pg/µl for the native analytes, each level with 5 pg/µl of the corresponding ^{13}C-labeled internal standard. The lowest linear correlation coefficient R^2 (linear fit) was determined for PCB 156 at 0.998. The respective correlation coefficients R^2 of all other congeners were 0.999 and higher.

Because of the high selectivity of the MS/MS technique, it was examined whether the extent of clean-up steps necessary for GC-HRMS determinations can be reduced in order to speed up the analysis time. For this, the usual clean-up of the non-ortho PCB fraction of an animal fat on a carbon column was omitted. The result is illustrated in Fig. 10. The left chromatograms show the sample without and the right chromatograms with carbon clean-up. The different retention times of the peaks are due to the use of two GC columns of different length.

The extract without carbon clean-up contains a lot of signals from co-extracts that may even pretend the occurrence of minor PCB concentrations and thus hampers the unequivocal determination of PCB 126. The clean-up removes the co-extracts and only shows the ^{13}C-labeled PCB 126. Although automatically integrated by the software, the concentration for PCB 126 is below the LOQ.

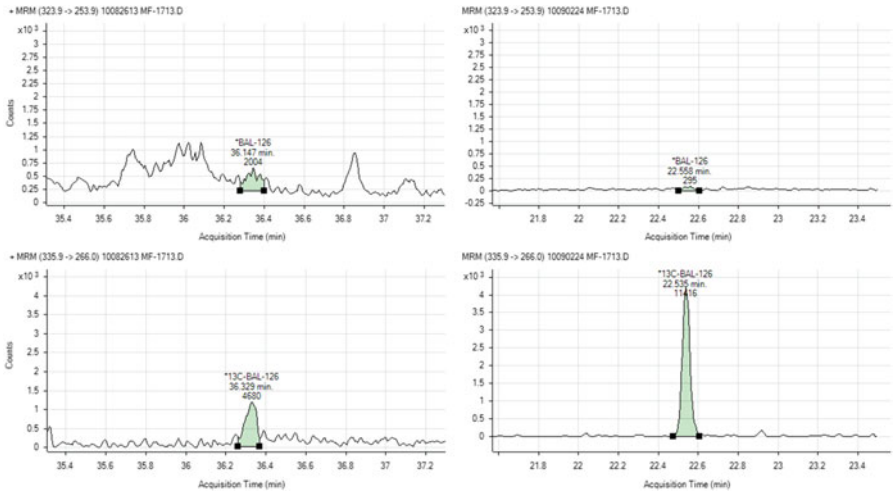

Fig. 10 Influence of carbon clean-up on the determination of PCB 126 (top: PCB 126, bottom: ^{13}C-PCB 126) in animal fat (left: without carbon clean-up, right: with carbon clean-up)

Following this and similar attempts, it was concluded that the extent of clean-up that is needed for GC-HRMS cannot be reduced for GC-MS/MS analyses.

The optimized MS/MS settings for the GC-MS/MS analysis of non-ortho and mono-/di-ortho-PCBs in feed and food are depicted in Tables 2 and 3. As the final PCB extracts in our in-house method are collected as two fractions by elution with different solvents, containing non-ortho-PCBs, and mono-ortho/di-ortho PCBs, respectively, the MS/MS settings are given in two tables. The MS/MS settings for the two PCB fractions are to the greatest possible extent similar. In all cases, each native analyte and its corresponding ^{13}C-labeled internal standard were measured using two precursor ions and two different product ions. The dwell times were 25 msec for all native PCB congeners, 125 msec for the ^{13}C-labeled non-ortho PCBs, and 75 msec for the ^{13}C-labeled mono-ortho- and di-ortho congeners. The MRM settings were each split into three time segments. The MS transfer line and ion source temperatures were each set to 280°C and the quadrupoles to 150°C. Nitrogen at 1.5 ml/min and Helium at 2.25 ml/min were used as collision cell gases.

The GC column was an HT-8, 50 m × 0.22 mm, 0.25 μm. Helium was used as carrier gas at a constant flow of 1.2 ml/min. The GC oven temperature program for the determination of non-ortho PCBs was as follows: 120°C (2 min), 40°C/min to 160°C (0 min), and 7°C/min to 300°C (10 min). The GC oven temperature program for the determination of mono-ortho/di-ortho PCBs was: 80°C (3 min), 20°C/min to 160°C (0 min), and 4°C/min to 300°C (8 min).

An automated liquid sampler with the sampler tray cooled to 5°C was used to make 2 μl pulsed cold splitless injections from both fractions.

Table 2 MS/MS settings for native non-ortho PCB congeners and their ^{13}C-labeled internal standards

TS	Segment start time (min)	Analyte	RT (min)	Quantifier		CE (V)	Qualifier		CE (V)
				Precursor	Product		Precursor	Product	
1	19.0	^{13}C-PCB 81	20.74	301.9	232.0	28	303.9	234.0	28
		PCB 81	20.75	289.9	220.0	28	291.9	222.0	28
		^{13}C-PCB 77	21.12	301.9	232.0	28	303.9	234.0	28
		PCB 77	21.13	289.9	220.0	28	291.9	222.0	28
2	22.0	^{13}C-PCB 126	23.55	335.9	265.9	28	337.9	267.9	28
		PCB 126	23.56	323.9	253.9	28	325.9	255.9	28
3	25.0	^{13}C-PCB 169	26.26	371.9	301.9	28	369.9	299.9	28
		PCB 169	26.27	359.9	289.9	28	357.8	287.9	28

Table 3 MS/MS settings for native mono-ortho and di-ortho PCB congeners and their ^{13}C-labeled internal standards

TS	Segment start time (min)	Analyte	RT (min)	Quantifier		CE (V)	Qualifier		CE (V)
				Precursor	Product		Precursor	Product	
1	22.0	^{13}C-PCB 28	24.34	268.0	198.1	26	270.0	198.1	26
		PCB 28	24.35	256.0	186.0	26	258.0	186.0	26
		^{13}C-PCB 52	25.66	302.0	232.0	28	304.0	234.0	28
		PCB 52	25.67	289.9	220.0	28	291.9	222.0	28
2	29.0	^{13}C-PCB 101	30.15	335.9	266.0	28	337.9	268.0	28
		PCB 101	30.16	323.9	253.9	28	325.9	255.9	28
		^{13}C-PCB 123	33.55	335.9	266.0	28	337.9	268.0	28
		PCB 123	33.56	323.9	253.9	28	325.9	255.9	28
		^{13}C-PCB 118	33.76	335.9	266.0	28	337.9	268.0	28
		PCB 118	33.77	323.9	253.9	28	325.9	255.9	28
		^{13}C-PCB 114	34.19	335.9	266.0	28	337.9	268.0	28
		PCB 114	34.20	323.9	253.9	28	325.9	255.9	28
		^{13}C-PCB 153	34.50	371.9	301.9	28	369.9	299.9	28
		PCB 153	34.51	359.8	289.9	28	357.8	287.9	28
		^{13}C-PCB 105	35.15	335.9	266.0	28	337.9	268.0	28
		PCB 105	35.16	323.9	253.9	28	325.9	255.9	28
		^{13}C-PCB 138	35.88	371.9	301.9	28	369.9	299.9	28
		PCB 138	35.89	359.8	289.9	28	357.8	287.9	28
		^{13}C-PCB 167	37.64	371.9	301.9	28	369.9	299.9	28
		PCB 167	37.65	359.8	289.9	28	357.8	287.9	28

(continued)

Table 3 (continued)

TS	Segment start time (min)	Analyte	RT (min)	Quantifier		CE (V)	Qualifier		CE (V)
				Precursor	Product		Precursor	Product	
3	38.5	^{13}C-PCB 156	38.78	371.9	301.9	28	369.9	299.9	28
		PCB 156	38.79	359.8	289.9	28	357.8	287.9	28
		^{13}C-PCB 157	39.06	371.9	301.9	28	369.9	299.9	28
		PCB 157	39.07	359.8	289.9	28	357.8	287.9	28
		^{13}C-PCB 180	39.17	407.8	337.9	30	405.8	335.9	30
		PCB 180	39.18	393.8	323.9	30	395.8	325.9	30
		^{13}C-PCB 189	42.43	407.8	337.9	30	405.8	335.9	30
		PCB 189	42.44	393.8	323.9	30	395.8	325.9	30

3 Results and Comparison GC-MS/MS vs. GC-HRMS

In order to evaluate whether GC-MS/MS has comparable capabilities as GC-HRMS for the unequivocal confirmation of dioxin and PCB levels in food and feed, more than 80 food and feed samples were processed with our in-house method and analyzed applying GC-HRMS on an AutoSpec Ultima and/or a DFS system at a resolution of $R = 10{,}000$. The same sample vials were then transferred to the GC-MS/MS system (Agilent 7000 GC-MS/MS) and analyzed by another operator who was not aware of the findings on the GC-HRMS.

In brief, the extraction and clean-up procedures are as follows [22, 23]: After spiking with ^{13}C-labeled internal standards, feed samples are extracted by means of Soxhlet with toluene/acetone (7/3). Following evaporation, the raw extracts are cleaned up on a sulfuric acid coated silica column from which the dioxins and PCBs are eluted with hexane. The separation of dioxins and PCBs was performed on a Florisil column (3% water). While the PCBs were extracted with hexane, the dioxins are eluted with toluene and subsequently further cleaned up on Carbopack C/Celite 545. Following the elution from the Florisil column, the PCB fraction is separated into non-ortho PCBs and mono-ortho/di-ortho PCBs on a Norit/Celite 545 column. After evaporation and reconstitution of the three fractions in toluene, the final volumes for GC separation were 15, 40, and 250 µl for dioxins, non-ortho PCBs, and mono-ortho/di-ortho PCBs, respectively.

The analytical procedure for food of plant origin generally follows the methodological set-up for feeding stuffs. For food of animal origin, the first step generally consists of the extraction of fat of which an aliquot of 2 g was then fortified with ^{13}C-labeled internal standards and transferred to the sulfuric acid coated silica column as described above. The further clean-up was performed as for feeding stuffs and food of plant origin. Recently, the manual clean-up was replaced by an automated procedure on a DEXTech™ system from LCTech [23] which resulted in a considerable speed-up of the clean-up and thus in an increased sample throughput.

3.1 Dioxins

Figure 11 shows the chromatograms of a GC-MS/MS analysis of an egg extract that was analyzed during a pollution incident where the chickens were fed dioxin contaminated feedingstuffs. The concentrations in this sample were determined as 15.5, 3.4, and 3.1 pg/g fat for 2,3,7,8-TCDF, 1,2,3,7,8-PCDF, and 2,3,4,7,8-PCDF, respectively. The chromatograms clearly demonstrate that the unequivocal confirmation of dioxin levels in the low pg/g fat range is feasible without problems.

Figure 12 shows the comparative results for 50 food and feed samples over the range from <0.05 up to 27 pg/g that were obtained by GC-HRMS and GC-MS/MS. All results in this and the following figures are presented as upperbound WHO_{1998}-TEQ values expressed as the percentage difference of the GC-MS/MS result

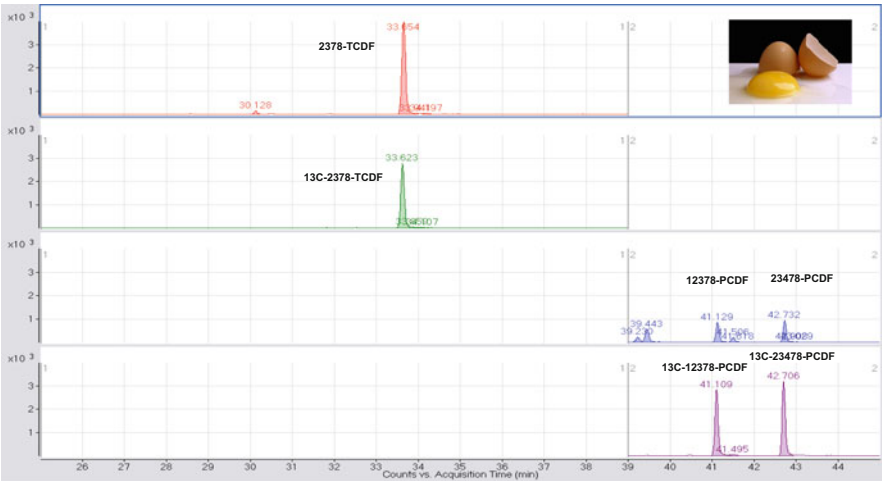

Fig. 11 Determination of 2,3,7,8-TCDF, 1,2,3,7,8-PCDF, and 2,3,4,7,8-PCDF based on their corresponding ^{13}C-labeled internal standards by GC-MS/MS in an egg extract. For clarity, only one transition is illustrated for native congeners and internal standards

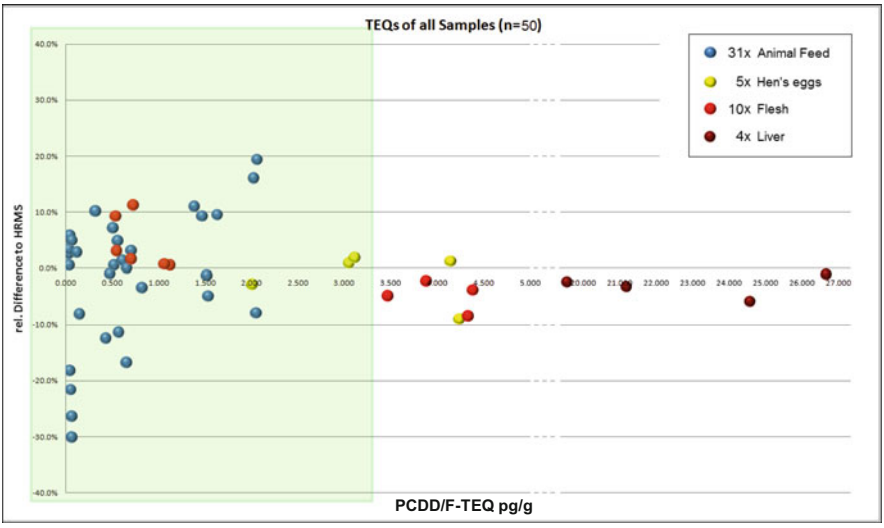

Fig. 12 Comparison of dioxin results (range <0.05–27 pg/g, upperbound WHO_{1998}-TEQs) for 50 feed and food samples analyzed by GC-MS/MS relative to the corresponding GC-HRMS values. Data on samples of animal origin are expressed on a fat basis, results for feed on 88% dry matter

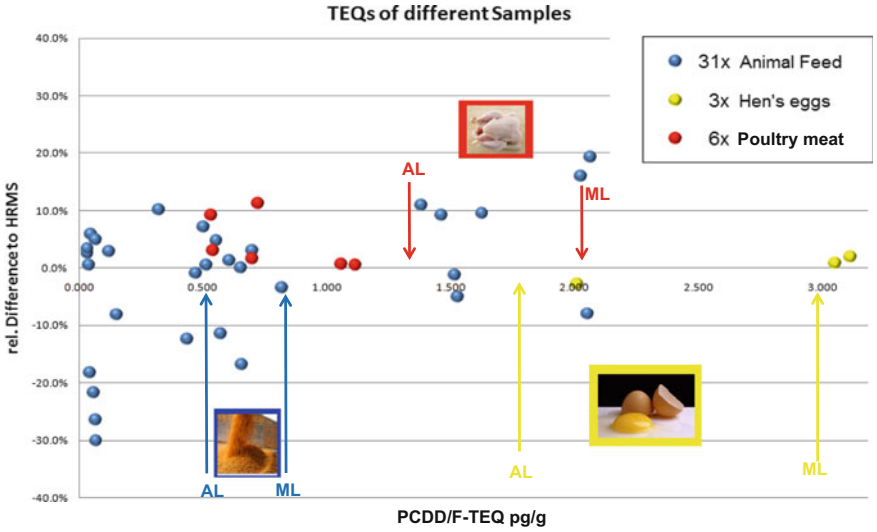

Fig. 13 Comparison of dioxin results (range <0.05–3 pg/g upperbound WHO_{1998}-TEQs) for 40 feed and food samples analyzed by GC-MS/MS relative to the corresponding GC-HRMS values. Data on samples of animal origin are given on a fat basis, results for feed on 88% dry matter

relative to the GC-HRMS data. While the data on eggs, meat, and liver are calculated on fat basis, the results for feed are based on 88% dry matter.

The shaded area in Fig. 12 between <0.05 and around 3 pg/g represents the concentration range where the legal maximum and action levels are stipulated. This range is expanded and depicted in Fig. 13 together with the then effective maximum and action levels for feedingstuffs, poultry meat, and chicken eggs. Food and feed samples that contain dioxin levels greater than 3 pg WHO_{1998}-TEQ/g revealed quantitative GC-MS/MS results that are within ±10% of the corresponding concentrations obtained by GC-HRMS. All samples that contained dioxins at concentrations below 3 pg WHO_{1998}-TEQ/g were generally within ±20% and thus show very good agreement between application of GC-MS/MS and GC-HRMS. Only some samples well below the respective maximum and action levels in the range of <0.05 pg/g gave relative differences up to−30%. These differences may be partially due to the fact that all results are expressed as upperbound concentrations taking the numerical value of the limit of quantification for the calculation of total TEQ values into account in those cases where a dioxin congener could not be detected. In contrast to GC-HRMS which gave a range of LOQs between 0.01–0.06 pg/g for the various congeners, the corresponding LOQs for GC-MS/MS were between 0.02–0.08 pg/g.

The data demonstrate the potential of GC-MS/MS for the confirmation of dioxin concentrations well below the maximum and action levels and thus the capability for unequivocally checking for compliance of feed and food commodities with legal limits.

3.2 PCBs

The same approach described for dioxins was also applied to the determination of PCBs, i.e., more than 80 food and feed samples that were initially analyzed by GC-HRMS were subsequently measured on the GC-MS/MS system by a different operator who was not informed on the results generated by GC-HRMS.

3.2.1 Dioxin-like PCBs

Figure 14 shows the GC-MS/MS determination of PCB 126 in a pig fat sample that was analyzed as part of a proficiency test organized by the European Reference Laboratory for Dioxins and PCBs. The left chromatogram shows the MRM transition m/z 323.9 → 253.9 which was used as the quantifier transition. In the right chromatogram, this transition is overlaid with the second measured MRM transition m/z 325.9 → 255.9 which serves as the qualifier MRM. The intensities of the two peaks match perfectly between the dotted lines which indicate the maximum permitted tolerance based on standards for the ratio of the relative intensities around the two transitions. The concentration for PCB 126 in this sample was determined as 2.85 pg/g fat.

Figure 15 shows the comparative results of 80 feed and food samples analyzed for DL-PCBs initially by GC-HRMS and subsequently by GC-MS/MS. While the data on food of animal origin are calculated on fat basis, the results for feed are based on 88% dry matter. The agreement between the results obtained for the WHO_{1998}-TEQ values obtained by the two techniques at levels above 1 pg/g was in almost all cases between ±10%.

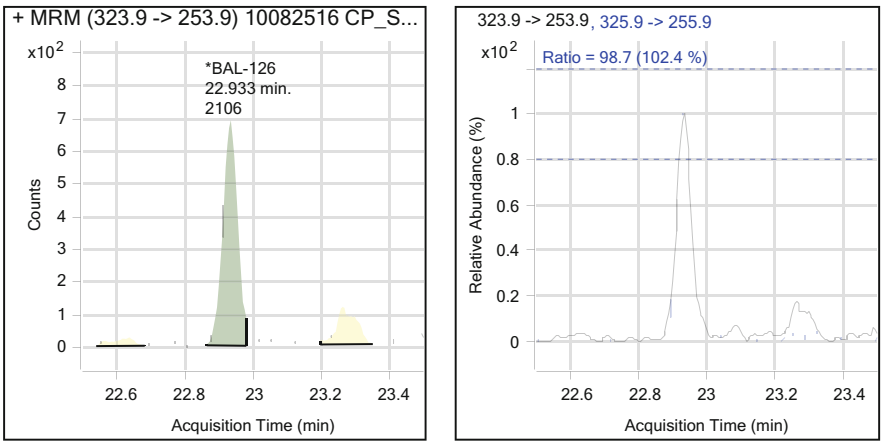

Fig. 14 Determination of PCB 126 in a pig fat sample by GC-MS/MS. (Concentration: 2.85 pg/g fat)

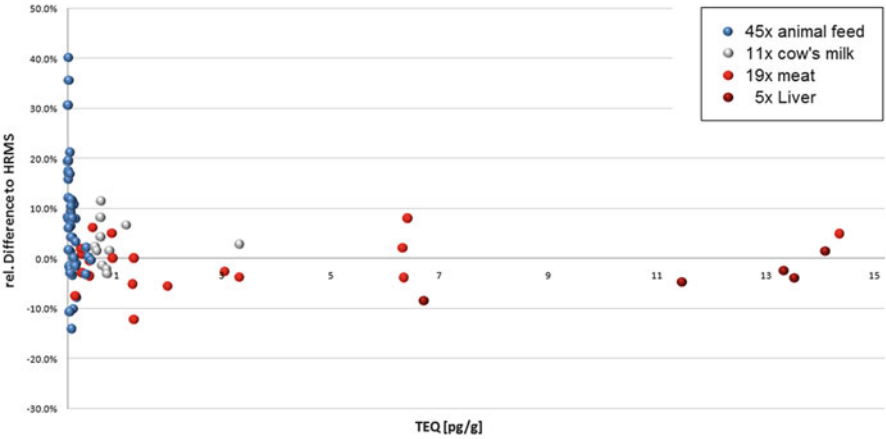

Fig. 15 Comparison of DL-PCB results (pg/g; upperbound WHO_{1998}-TEQs) for 80 feed and food samples analyzed by GC-MS/MS relative to the corresponding GC-HRMS values. Data on samples of animal origin are given on a fat basis, results for feed on 88% dry matter

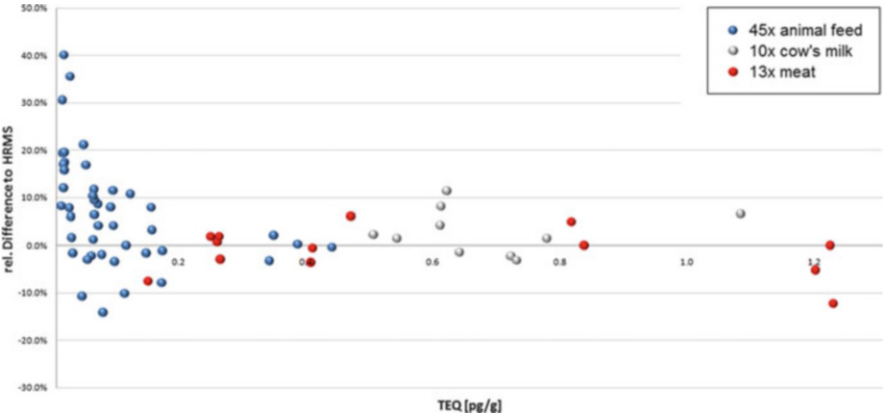

Fig. 16 Comparison of DL-PCB results (pg/g; upperbound WHO_{1998}-TEQs) for 68 feed and food samples that gave concentrations less than 1.2 pg/g analyzed by GC-MS/MS relative to the corresponding GC-HRMS values. Data on samples of animal origin are given on a fat basis, results for feed on 88% dry matter

As the concentration range below 1 pg TEQ/g is of special importance for checking of compliance with legal limits, this range was expanded and is depicted in Fig. 16. This figure demonstrates the good agreement of the results obtained by GC-HRMS and GC-MS/MS even down to concentrations of 0.1 pg/g with a percentage difference of ±15%. Only those feed samples with DL-PCB levels below 0.1 pg WHO_{1998}-TEQ/g gave some results with percentage differences between −15 and +40%.

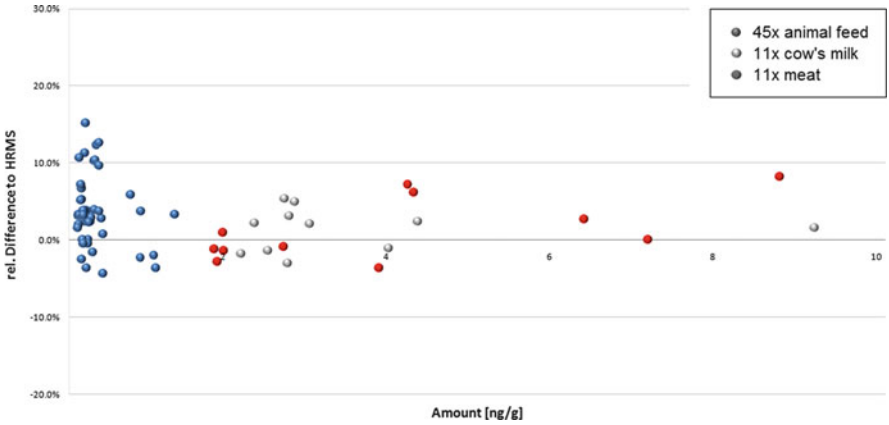

Fig. 17 Comparison of NDL-PCB results (sum of 28, 52, 101, 138, 153, and 180) for 67 feed and food samples analyzed by GC-MS/MS relative to the corresponding GC-HRMS values. Data on samples (ng/g upperbound) of animal origin are given on a fat basis, results for feed on 88% dry matter

3.2.2 Non-Dioxin-Like PCBs

Figure 17 shows the comparative results (upperbound) for the sum of the six NDL-PCBs 28, 52, 101, 138, 153, and 180 in 68 food and feed samples measured by GC-MS/MS and GC-HRMS. While the results for the feed samples are depicted as ng/g based on 88% dry matter, the data for the food samples are given as ng/g fat. All data are expressed as the percentage difference between the results obtained by GC-MS/MS relative to the GC-HRMS analyses. For the presentation, the range up to 10 ng/g was chosen as the EU Commission initially intended to set maximum levels for certain foodstuffs at this concentration. The agreement between the sum of the six NDL-PCBs generated by GC-HRMS and GC-MS/MS at levels between 0.5 and 10 ng/g is within the range of ±10%. Only some feed samples with upperbound concentrations below 0.5 ng/g gave percentage differences greater than +15%. However, these levels represent the environmental background contamination with NDL-PCBs and thus are not relevant in case regulatory limits have to be checked for compliance. Meanwhile, the lowest maximum levels for the sum of the six NDL-PCBs in food were set at 40 ng/g fat by the EU Commission [3]. This level can easily be met by the application of GC-MS/MS.

4 Conclusions

Due to improved sensitivity because of enhanced ion source optics and increased ion yield, application of GC-MS/MS for the determination of dioxins and PCBs in feed and food has become a powerful and cost-effective alternative to GC-HRMS.

Modern GC-MS/MS systems nowadays provide linear, reproducible, and sensitive detection of these contaminants down to low fg/μl levels and thus enable a reliable and unequivocal surveillance of regulatory limits. A comparison of analytical results obtained by GC-MS/MS and GC-HRMS for a number of feed and food commodities around their legal limits generally gave relative differences below ±15%. A number of proficiency tests organized by the EU-RL and other providers have proven the suitability of GC-MS/MS for the routine determination of low dioxin and PCB levels in feed and food at the levels of interest laid down in the European legislation. This resulted in the inclusion of GC-MS/MS into European feed and food legislation as an appropriate confirmatory method equivalent to GC-HRMS [19, 20].

Acknowledgements The meticulous processing of the food and feed samples by Ludger Wessel, Matthias Keitlinghaus, and Markus Stening as well as the skillful operation of the GC-HRMS and GC-MS/MS systems by Lothar Bathe and Ursula Möhlenkamp are gratefully acknowledged. We are also grateful to Chris Sandy for his great support in setting up the GC-MS/MS system and his valuable technical advice.

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Levels and Trends of Dioxins, PCBs, and Other POPs in Abiotic Compartments

Richard J. Wenning and Linda B. Martello

Abstract Studies reporting on levels of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), polychlorinated biphenyls (PCB), and other persistent organic pollutants (POPs) in the air, soil, sediment, and surface water are available from the 1970s to the present. While typically focused regionally, these studies provide important information on the evolution of the distribution and occurrence worldwide of environmental contamination attributed predominantly to human activity. This chapter summarizes monitoring work conducted during the past four decades that have contributed to our understanding of levels and trends in the abiotic environment. This includes a summary of available environmental data from the past two decades describing the current understanding of background conditions and global cycling of POPs. Data are summarized at a continental level for Africa, Asia/Pacific, Europe, North America and South America, and the polar regions. The results confirm the early views of the preeminent scientist in this field, Dr. Otto Hutzinger, who first suggested a "pulse" of highly persistent compounds entering the environment beginning in the 1930s and 1940s, peaking in the 1960s and 1970s, and gradually declining to the present time. This trend, however, does not yet apply to emerging POPs or to some regions of the world where long-range transport processes and monitoring work are evident only more recently. Overall, the distributions and levels of POPs continue to behave as predicted by Dr. Hutzinger; environmental levels of classical industrial chlorinated compounds are generally higher in the northern hemisphere than in the southern hemisphere, and levels are declining worldwide with the possible exception of PCBs and in remote locations where global cycling is finally extending its influence.

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Dr. Hutzinger's pioneering research over the past half-century laid the foundation for ongoing improvements in laboratory analysis and monitoring and interpretation of complex data sets, which continue to close the knowledge gap and improve our understanding of POPs in the air, soil, sediment, and water around the world.

Keywords Abiotic compartments, Air, Marine environment, Persistent organic pollutants, POPs, Sediment, Soil, Surface water, Terrestrial environment

Contents

1 Introduction

In 1984, Dr. Otto Hutzinger observed that the interaction of chemicals with biological organisms was as old as life itself, but that chemical pollution was human interference with natural chemical cycles and the release of man-made, unnatural compounds [1]. According to Dr. Hutzinger, the challenges posed by air pollution, water pollution, occupational exposure, pollution from agricultural practices, and contamination of food were best understood from a historical perspective, which could aid in the rational and prudent evaluation of present-day pollution problems.

Over 20 years from 1984 to 2004, Tickner et al. [2] concluded from a review of sustainable chemistry practices, which Dr. Hutzinger encouraged many years ahead of its time, that data collection on chemical risks and phaseouts of the most egregious chemicals alone would not promote the cultural and institutional changes needed to ensure the design and implementation of safer chemicals, processes, and products in the future. While society has benefited greatly from the commercial use of a growing class of persistent organic chemicals, some of those benefits have come at great cost to the quality of the environment.

For example, despite our efforts to restrict the commercial use of polychlorinated biphenyls (PCBs) and reduce incidental formation of polychlorinated dibenzo-p-dioxins (PCDDs or dioxins) and polychlorinated dibenzofurans (PCDFs or furans) in various combustion and chemical manufacturing activities, the contamination of soil and sediment and transient levels in ambient air and surface water continues to the present day. There is conflicting evidence from human and biota monitoring studies about whether current levels are indicative of a

continued decrease from the generally higher levels observed in the 1970s and 1980s or reflect a plateau associated with either continued (albeit low) inputs or redistribution and recycling in the environment [3].

Our understanding of POPs in the abiotic environment emerged from environmental chemistry research and monitoring of polychlorinated biphenyls (PCBs), dioxins, and certain chlorinated pesticides widely used or accidentally released in the 1950s to 1970s. The 419 theoretically possible individual PCB, PCDD, and PCDF congeners have a range of physicochemical characteristics [4–6], which profoundly affect their persistence and environmental distribution, as well as bioaccumulation potential [7, 8]. PCDD/PCDFs were never produced intentionally for chemical manufacturing or marketable purposes. Dioxins were generated from many different combustion processes wherein a source of chlorine and ringed compounds was heated to a certain temperature range [9]. Dioxins also occurred as unwanted by-products in chemical manufacturing processes and various chlorinated chemical formulations. Among the best recognized sources of dioxins are chemical processes associated with the manufacture of chlorinated phenols such as 2,4,5-trichlorophenol and pentachlorophenol, pesticides such as Agent Orange, and antibacterial formulations such as hexachlorophene [9].

The leadership of environmental scientists such as Dr. Hutzinger and others over the past few decades has led to many important advances in our understanding of long-range transport and global cycling of man-made pollutants in the abiotic environment. Their leadership also has guided several improvements in sampling and laboratory analytical methods supporting environmental monitoring and advanced our understanding of the implications to human health and ecology. These and other scientific advancements have prepared us well for the new challenges posed by emerging persistent chemicals [10].

The purpose of this chapter is to build on monitoring work conducted from about the 1970s, when scientists first began to raise concerns about the persistence and toxicity of so-called “classical” industrial chlorinated chemicals appearing in the environment, namely, the PCDDs, PCDFs, PCBs, and a few chlorinated pesticides such as DDT and chlordane. Monitoring and laboratory work evolved significantly from the mid-1980s through leadership and technical advancements by Dr. Hutzinger and others. A closer inspection of current levels and trends and understanding of background conditions and global cycling of POPs are provided from reflection on these earlier decades and closer examination of available environmental data from the past 10 years. Data are summarized at regional and continental levels for Africa, Asia/Pacific, Europe, North America and South America, and the polar regions.

Collectively, the information from nearly 50 years of monitoring and analysis allows us to draw conclusions about levels and trends in air and the terrestrial (i.e., soils) and aquatic (i.e., sediments and surface water) environments around the world, as well as forecast likely levels in the future. This effort draws heavily from environmental surveys conducted by research organizations that have been actively involved in POPs monitoring and research activities in several countries, including the polar region. In many respects, this work is an update nearly 20 years

later of the temporal trend analysis reported by Alcock and Jones [11], which provided a similar perspective in the mid-1990s on levels in different abiotic environmental compartments.

2 Fate and Transport Characteristics

The generalized environmental processes by which persistent man-made chemicals such as the dioxins, furans, PCBs, chlorinated pesticides, and other persistent contaminants move through the environment are reasonably well known [1, 12]. A global distribution model has been proposed to explain the accumulation of these substances in environmental compartments at higher latitudes [13]. Global distillation theory is based on physical–chemical POP properties, sources to the environment, and the earth's climatic conditions (Fig. 1). The physical and chemical properties that control the behavior of POPs are their low vapor pressures, low solubility in water, and preferences for binding to organic matrices. POPs are man-made organic compounds that, to a varying degree, resist photolytic, biological, and chemical degradation. POPs are also semi-volatile, enabling migration across long distances in the atmosphere before deposition.

In the atmosphere, POPs generally exist in both the gaseous phase and bound to particles, depending upon the environmental conditions [14]; two particularly important partitioning variables are air temperature and total suspended particle loading [15]. For dioxins and similar chlorinated compounds, there is a continual exchange between the particle and vapor phase and during the summer months, and when temperatures are high, the less chlorinated compounds tend to be found

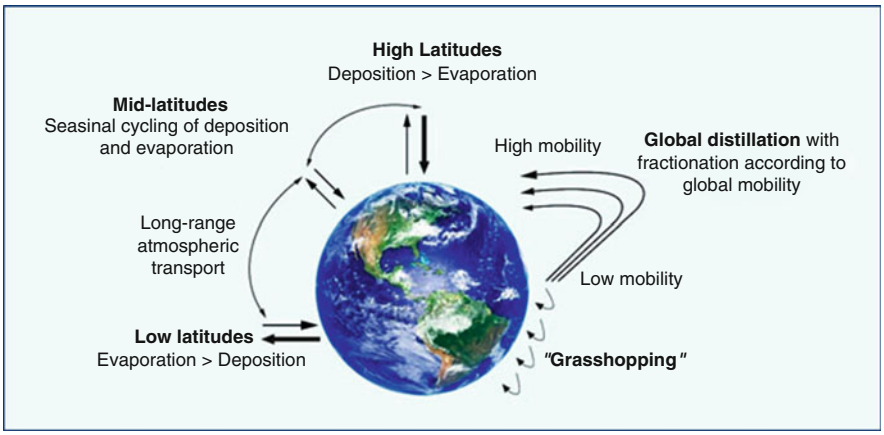

Fig. 1 The global distillation theory has been proposed to explain the distribution of POPs worldwide and particularly their occurrence in remote regions around the world where human activity is minimal (adapted from European Union Science Education Multimedia repository; http://www.eusem.com/main/CE/SIP_C2_bg)

predominantly in the vapor phase; in the winter months, dioxins and other compounds are split between the particulate and vapor phases [16]. In the polar region, snowpack can also influence atmospheric behavior [17, 18]. Similarly, seasonal differences have been noted in the exchange of POPs across the air–water/soil interface [19].

The future is somewhat uncertain, however; some suggest that indirect consequences of climate change such as shifts in agriculture and urbanization are more likely to influence future contaminant distributions than direct climate change [20]. Others suggest changing weather patterns and hydrological conditions will be more important than human activity, particularly for polar and remote regions [21, 22]. Modeling to predict fate and cycling in the North Sea also indicates that climate change may have a negligible influence on the fate and transport of PCB 152 and HCH [23] into the twenty-first century.

The main pathway by which POPs move from the atmosphere to the terrestrial environment is deposition to soil, vegetation, and surface waters by wet and dry processes. Vapor phase and particle-phase dry deposition are important for deposition to soil, though organic matter content is important to long-term retention in the soil matrix [24, 25]. Small amounts can be returned to the atmosphere by the resuspension of previously deposited material or re-volatilization in the case of lower halogenated substances [26]. It is well understood that POPs accumulate in most soil types [24], even clay soils devoid of significant organic content [27]. Half-lives for degradation of dioxins, PCBs, and most persistent organics in soil are generally measured in years [28], and both aerobic and anaerobic degradation of higher to lower chlorinated congeners remain uncertain [29, 30].

Air–vegetation exchange of POPs is an important process controlling the entry of POPs into terrestrial food chains and the air–vegetation transfer influenced by several physiochemical and environmental factors and plant characteristics [31]. Levels in vegetation tend to be surficial and typically reflect recent atmospheric deposition since most vegetation is exposed for relatively short periods of time, with new growth replacing old and crops being harvested. For agricultural leaf crops, the main source of contamination is direct deposition from the atmosphere and soil splash and for root crops soil contamination and binding to the lipids in cell walls [32]. However, the significance of root uptake of POPs from the soil requires further investigation, as there appear to be large differences between plant species [32, 33]. Grazing animals are exposed to POPs by ingesting contaminated pasture crops, whereby these substances are found to accumulate primarily in the fatty tissues and milk [34].

In the aquatic environment, the major inputs of POPs to surface water bodies are via atmospheric deposition and direct inputs from industrial effluent and soil runoff. It is well established that POPs partition rapidly to organic matter and accumulate in sediments, sometimes permanently but often times temporarily and thereby becoming a future source of contamination elsewhere or affecting aquatic organisms and human health through the diet [35, 36]. Dissolution in surface water is generally not a significant pathway, though concerns about rising temperatures could alter partitioning of POPs in sediments and biota [37]. POPs accumulate in aquatic

biota as a result of the ingestion of contaminated organic matter and through the movement of substances through food chains. The concentrations of persistent substances in fish tissue is generally found to increase up the food chain as a result of the progressive ingestion of contaminated prey [38], although the processes by which this occurs are not well quantified, at present, in some deep water, tropical, or remote ecosystems [39, 40].

3 Background Levels

Considerable work has been conducted over several decades to understand background levels in the environment, predicated on the assumption that dioxins and other POPs have both a natural and anthropogenic origin. There is little debate that the majority of POPs are from anthropogenic origins in the northern hemisphere [41]. In practice, specifically in the regulatory community, the term background requires clarification if research and environmental regulation is to distinguish between the natural occurrence of some POPs and their distribution in the environment from diffuse man-made sources, both of which are characterized by large area extents, low concentrations, and the lack of point sources.

Increasingly in the context of monitoring POPs, terms such as background, background soils, and background content or concentration typically refer to widespread pollution caused by long-range transport or recycling of persistent substances alternately trapped and released from different soil and water compartments [20, 42]. An intuitive approach to allocate background locations is chosen by Rotard et al. [43]. A more recent study shows that the understanding of background condition is only possible if there is a sufficiently large data set to distinguish with statistical confidence differences between background diffuse pollution and local or regional contamination [44].

Several studies, however, suggest that several chlorinated and brominated compounds, including certain dioxins and PBDEs, can be formed by natural processes [45, 46], thereby supporting the theory of background levels in air, sediments, soil, and surface water in different parts of the world. Of particular scientific interest is the presence of dioxins found in sedimentary kaolin clay in North and South America, Europe, the South Pacific, and Asia [27, 47]. Schmitz et al. [48] confirm Horii et al. [47] observations that dioxin levels are higher in tertiary, primary nonsedimentary kaolin (ball) clays than in secondary, sedimentary kaolinitic and lignitic clays, suggesting an as yet unknown geologic enrichment process occurs in the environment. The available evidence remains somewhat inconclusive, and scientists involved in this work do not extend the theory to PCBs, chlorinated pesticides, the vast majority of halogenated compounds, and emerging POPs, which are generally assumed to be entirely of human origin.

The challenges associated with the determination of background environmental levels from monitoring conducted in rural or remote areas are well highlighted by work conducted on the dioxins. A summary of PCDD/PCDF levels in rural or

Table 1 Total PCDD/PCDFs and dioxin-like PCBs (dl-PCB) in surface soil from rural or remote regions

Country/region	Soil environment	Date	*N*	Σ PCDD/PCDFs (pg g^{-1} dw)	Σ dl-PCBs (pg g^{-1} dw)	PCDD/PCDF TEQ (pg TEQ/g)	PCDD/PCDF + dl-PCB TEQ (pg TEQ/g)	TEQ scheme	Reference
Arctic (Ny-Ålesund)	Tundra soil	2008	20	9.97 (3.55–16.6)	–	0.33 (0.16–0.62)	–	WHO 1998	[49]
Antarctic (Fildes Peninsula)	Tundra soil	2007–2008	15	2.18 (0.49–6.72)	–	0.02 (ND–0.06)	–	WHO 1998	[49]
Australia	Outback soil	2003	19	890 (0.31–15,000)	5.1 (ND–66)	0.38 (0.00056–5.0)	0.38 (0.00068–5.2)	WHO 1998	[50–52]
Austria	Forest soil	1993	25	319 (106–2676)	–	4 (1.6–31.0)	–		[53]
Central Europe Alps	Spruce forest, humus layer	2004	31	313.84 (115.7–758)	1,591 (439–3,266)	4.44 (1.37–10.81)	6.76 (116.4–15.73)	WHO 1998	[54]
China (Zhangmu-Nyalam, Tibetan Plateau)	Grassland and forest soil	2011	9	26.22 (2.43–73.28)	–	0.37 (0.06–0.65)	–	WHO 1998	[49]
Italy (NW Lombardy region)	Agrarian soil	2011–2012	10	–	–	2.13 (0.38–5.27)	2.87 (0.43–5.49)	WHO 2005	[55]
Norway	Woodland soil	1998	21	560 (15–4,100)	–	10 (0.2–78)	–	WHO 1998	[56]
The USA	Background, rural soil	1985–2011	Data from 14 studies	–	–	1.1–7.1 (0.1–22.9)	–	WHO 2006	[57]

Data presented as mean (range). The Σ PCDD/PCDFs data represent the mean and range for reported measurements of all dioxin and furan congeners

remote soils typifies the wide range of so-called background conditions reported worldwide (Table 1). A detailed review of dioxins prepared by the US EPA [58] concluded that the background level in US soils was approximately 8 parts per trillion (ppt) of total dioxin equivalents (TEQ). USEPA's findings were based on 95 soil samples collected throughout the continental USA. More recent studies corroborate USEPA's findings, although data quality challenges continue to hamper clear understanding of natural occurrence and long-range transport from human sources and deposition in remote areas. Summarizing the available background soil data, Urban et al. (2014) concluded there was substantial variability in how soil dioxin data are presented in the literature (e.g., raw vs. summary data, congener vs. TEQ concentration, and number of congeners included in derivation of total level or TEQ). According to Urban et al. (2014), the reinterpretation of available data indicates that background levels in urban/suburban soils are higher and more variable than in rural soils: ranging 0.1 to 186 ng/kg TEQ and 0.1 to 22.9 ng/kg TEQ, respectively. Since much of the available data does not include dioxin-like PCBs, background TEQ levels in soil may be underestimated.

In Canada, 4 ng TEQ kg^{-1} (using WHO TEFs) is considered representative of the mean background concentration of PCDD/PCDFs in Canadian soils [59]. This value, however, may not accurately reflect the ambient background concentration of PCDD/PCDFs in soils elsewhere in Canada. For example, ambient PCDD/PCDF concentrations in soils collected from remote northern sites that were at least 20 km from human activity ranged from non-detectable to 0.000009 ng TEQ kg^{-1} (or 9 fg TEQ kg^{-1}) [59]. In general, scientists believe that natural background levels in extreme polar regions for all POPs are zero, and low (less than 1) ng/g soil levels are ambient levels reflecting either direct human activity or evidence of global cycling [60]. Meijer et al. [61] and Aichner et al. [62] describe how mechanisms such as vegetation and fate in different soil horizons likely influence soil levels of POPs.

Fewer and smaller data sets describing background soil conditions are available outside of North America [63]. According to Müller et al. [50–52], background concentrations of dioxin-like chemicals in Australian soils (0.54 to 3.8 pg TEQ g^{-1} dw) are, on average, among the lowest reported in any industrialized country; interestingly, soil from 1925 contained detectable concentrations of PCDDs, PCDFs, and PCBs at levels higher than in soil from the 1930s and 1940s. Buckland et al. [64] reported background dioxin concentrations of PCDD/PCDF expressed as I-TEQ ranging from about 0.17 to 1.99 pg g^{-1} dw in seven forest soils and five grassland soils collected in remote or pristine locations in New Zealand. The New Zealand results are somewhat similar to those reported in Australia, if results are expressed on a TEQ basis. In Germany, Rotard et al. [43] reported background concentration PCDD/PCDF in different cultivation types (forest, grassland, and plowland) ranged from about 10 to 110 pg I-TEQ g^{-1} dw. In Korea, Im et al. [65, 66] found 0.2 pg TEQ g^{-1} dw in mountaintop soil. In Brazil, Braga et al. [67] reported a concentration of 0.04 pg TEQ g^{-1} dw (PCDD/PCDF) in forest soil considered pristine. Work in the UK, Norway, and Europe indicates that

background levels tend to decline from south to north in soils and levels are higher in forests than grasslands [68, 69].

In the air, data from Cleverly et al. [70] continue to reflect current understanding. Cleverly et al. [70] reported preliminary results of air monitoring at 17 rural stations and eight national parks in the USA conducted four times during calendar year 2000. Two of the 17 stations were located in suburban Washington DC and San Francisco, CA, to provide an indication of levels in more populated areas. All of the 2,3,7,8-substituted dioxin congeners were detected in ambient air at the 15 rural stations; dioxins were detected in 70% of the air samples. Excluding PCB 169, PCBs also were detected in all air samples. There was a 28-fold range in dioxin-TEQ annual average air concentrations at the rural sites, ranging between 2.5 and 58.3 fg m^3. PCB-TEQ air concentrations ranged from 0.2 to 9.9 fg m^3, an approximately 50-fold range. Passive air sampling in Europe corroborates much of the observational data in the USA regarding regional differences attributable to urban and remote locations [71].

Work conducted to date on background levels of dioxins in soil and air demonstrates how background may change from area to area within and between regions. Although global averages are of general use, no specific global background levels, for example, in soils, can be defined for environmental management or health assessment purposes; at best, regional or local operational estimates can be made, though with caveats [72].

4 Levels in the Air

Atmospheric transport has long been recognized as a major mechanism for dispersion of dioxins, PCBs, and other POPs around the world and, as such, has been a particularly important focus of investigation for decades [24, 25]. Early work on atmospheric transport and the influence of highly populated regions on levels in the air initially focused on the US Great Lake region [73, 74, 171], UK [75, 76], and Europe [61, 77] (Fig. 2).

At present, the Global Atmospheric Passive Sampling (GAPS) Network is the only global-scale air monitoring and surveillance program for legacy POPs and new priority chemicals. The program involves periodic deployment of polyurethane foam (PUF) disk passive air samplers to approximately 60 locations around the world and testing for dioxins, organochlorine pesticides, PCBs, PBDEs, and emerging candidate POPs [79, 80]. Led by researchers from Environment Canada, the monitoring is intended to provide spatial and temporal (including seasonal) air concentration data useful to developing emission estimates, validating predictions from fate and transport models, and risk management decision-making [168].

GAPS results from PUF samplers deployed in 2002 for 2–7 months [79] and in 2005 for four consecutive 3-month periods [81] reveal similar results. Pozo et al. [81] reported that annual geometric mean concentrations in the air were

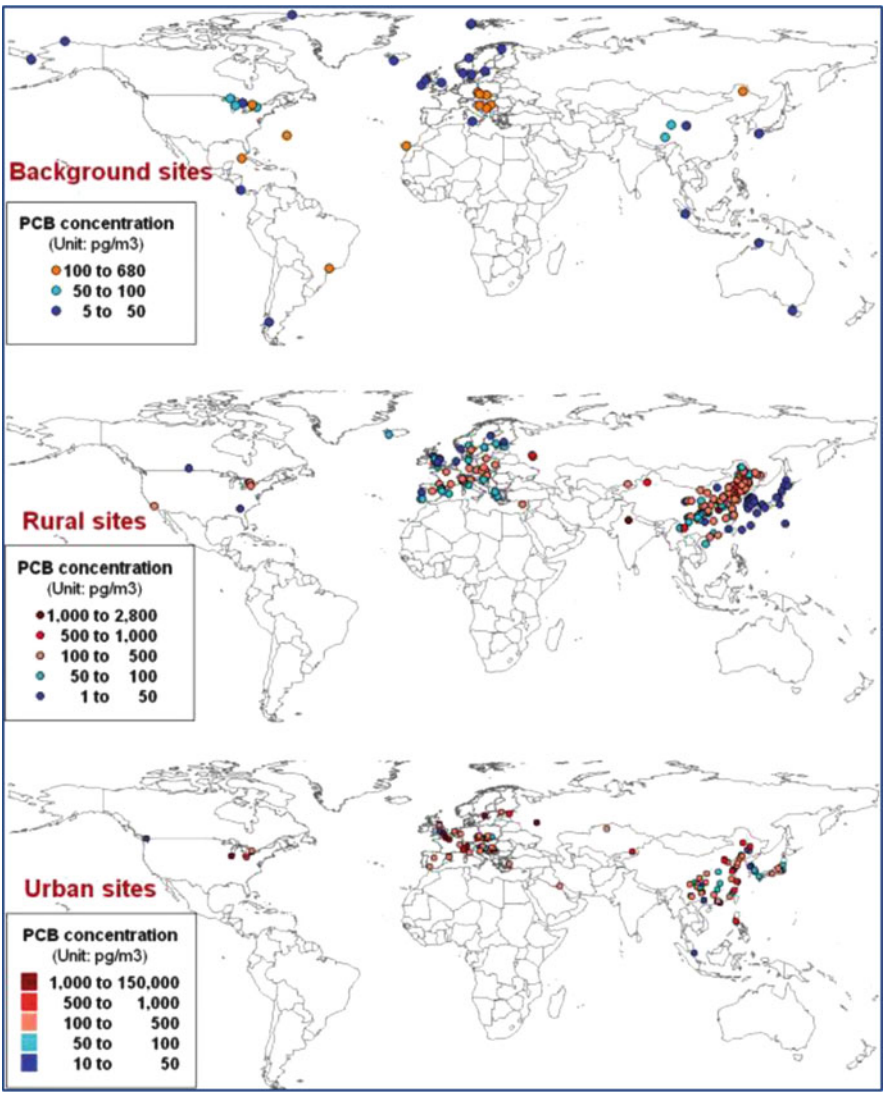

Fig. 2 Concentrations (pg/m^3) of ∑PCBs in ambient air reported at remote locations (*top*), rural sites (*middle*), and urban locations (*bottom*) around the world (adapted from [78])

highest for endosulfan (geometric mean, 82 pg/m^3) and PCBs (geometric mean, 26 pg/m^3); other chemicals regularly detected included α- and γ-hexachlorocyclohexane (HCH), chlordanes, heptachlor, heptachlor epoxide, dieldrin, *p*,*p*′-DDE, and PBDEs. Monitoring results from 2002 to 2009 did not reveal seasonal patterns on a global basis, although trends and seasonal patterns were evident for some substances. Endosulfans, for example, exhibit strong seasonality with highest concentrations during summer months at or near agricultural areas;

highest concentrations of POPs generally occur in the midlatitudes of the northern hemisphere; and PCB global emission estimates correlate well with the highest concentrations in developed and industrialized regions.

At the global scale, long-term monitoring data suggesting POP levels that may be declining overall [82] is optimistic and remains largely inconclusive for most scientists. Concerns about climate change and evidence of increases in the levels of several traditional and emerging POPs in marine food webs have prompted monitoring work in the Arctic in the past decade [83–85]. Air monitoring in the Arctic [86–89] and results from the third phase (2003 to 2011 monitoring) of Canada's Northern Contaminants Program (NCP) provide some evidence of declining trends for some but not for all POPs [90]. Bidleman et al. [83] found annual differences in the partially degraded fractions (enantiomer fraction) of HCH and chlordanes measured from 1994 to 2000 at the Alert, Canada, monitoring station, suggesting different emission sources contributing to atmospheric concentrations in the warm versus cold seasons.

POP contaminant trends are not apparent, however, in several developing countries where economic pressures may compromise some environmental regulations [91]. For much of the world, information is incomplete at the regional scale, and work is just beginning to assess spatial and temporal changes in the air [92, 93]. Work underway to train laboratories in passive air sampling for POPs in West, East, and South Africa, Latin America and the Caribbean, and Pacific Islands [94] will greatly help to fill this important knowledge gap. Initial ambient air results from this effort and reported by Bogdal et al. [95] suggest ∑PCBs are approximately four times higher in Africa (median 84 pg/m^3) than in the Pacific Islands or Latin America; ∑DDT is nearly six times higher in the Pacific Islands (306 pg/m^3) than in Africa and barely detectable in Latin America; and ∑PCDD/PCDF TEQs are slightly higher in Latin America (74 fg WHO98 TEQ/m^3) than in Africa and nearly 30 times higher than in the Pacific Islands (Fig. 3). E-waste and ship dismantling in Africa, malarial control programs in the Pacific Islands, and intensive urbanization in Latin America may be important factors contributing to the highest levels of PCBs, DDTs, and PCDD/PCDFs, respectively, observed in these regions.

In China, which arguably has the world's most notable air pollution, few surveys of the concentrations of POPs in the atmosphere on a national scale have been carried out so far, and, therefore, chemistry data concerning the overall contamination status of atmospheric POPs in China are absent. Zhao et al. [96] have proposed the use of tree bark as a passive sampling medium to understand regions acting as possible sources and sinks and global cycling because tree bark accumulates both gas-phase and particle-phase POPs simultaneously from the surrounding air [97] and reflects time-integrated overall air pollution levels [98]. By examining the spatial distribution of 18 polycyclic aromatic hydrocarbons (Σ18PAHs), five organic chlorinated pesticides (Σ5OCPs), ten polychlorinated biphenyls (Σ10PCBs), and 17 brominated flame retardants (Σ17BFRs) in 163 bark samples from 68 locations across mainland China, Zhao et al. [96] could demonstrate that

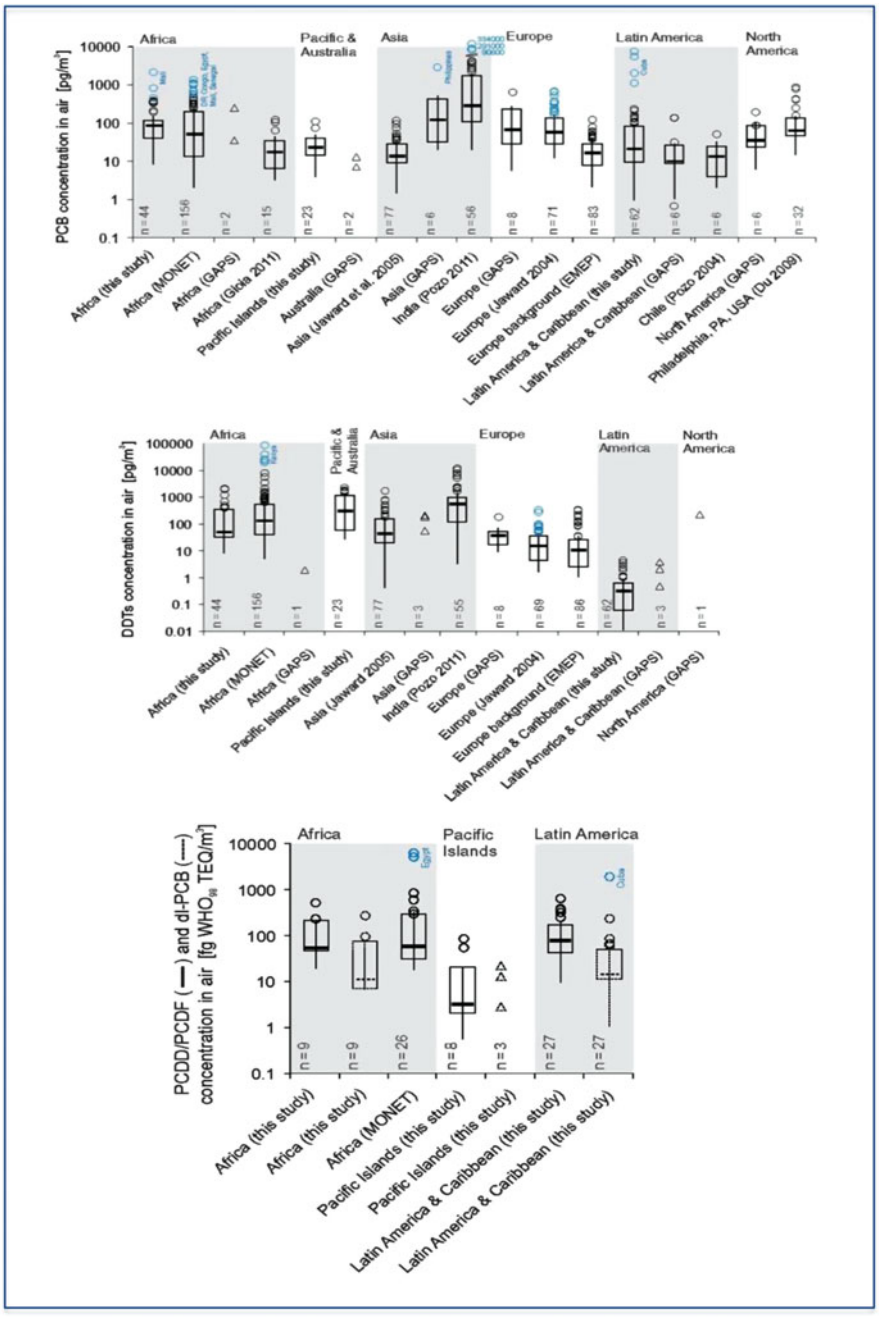

Fig. 3 Results of passive air sampling for ∑PCBs (pg/m^3), ∑DDTs (including *o*,*p*′ and *p*,*p*′ congeners of DDT, DDD, and DDE; pg/m^3), and ∑PCDD/Fs (fg WHOTEQ/m^3) in Africa, Pacific Islands, and in Latin America and the Caribbean (adapted from [95])

environmental contamination by atmospheric POPs was more serious in eastern and middle China than in western China.

Kalantzi et al. [99] argued that passive air sampling and the use of surrogate measures of air concentrations are sensitive to local, regional, and global-scale spatial and temporal atmospheric trends for many POPs and proposed butter and possibly other dairy products as useful monitoring surrogates. For most surrogate materials, confounding factors limit the usefulness of the data for monitoring or to predict long-term trends. Using dairy products as tool for air monitoring, for example, it has been shown that climatic factors and livestock management practices likely influence air–milk fat transfer processes [100–102]. Other surrogates such as vegetation also have limitations. For example, the collection and analysis of vegetation samples, such as conifer needles, lichens, and tree bark [103–105], may be hampered by specie differences, changes to predominant weather patterns, and other confounding factors that make data interpretation difficult [106, 107]. These and other factors confounding the use of either biotic or abiotic monitoring surrogates highlight the importance of assessing the spatial and temporal variability in long-term trend analysis [102, 108].

5 Levels in Terrestrial Environments

Generally, soil studies conducted during the 1960s to 1990s focused primarily on inputs from hazardous waste sites, industrial activities, and municipal sources. Scientific attention largely shifted beginning in the 2000s to understanding environmental fate and global distribution with increasing recognition that levels of dioxins, PCBs, and other POPs were different in urban, rural, and remote locations [11], as well as the advent of national POP inventories and concerns with both chemical persistence and endocrine disruption [109].

Several notable studies conducted during the past two decades provide a good understanding of spatial and temporal changes in soil levels of POPs worldwide. In general, ΣPCDD/PCDF TEQ, ΣPCB, and ΣPBDE levels in soils in North America, China, and Europe are 10–100 times higher than soil levels in Australia/New Zealand, Africa, and Latin America [12]. Further, it is widely acknowledged that the dioxins, PCBs, and several other legacy POPs are highest in heavily populated regions of the world [12]; for example, the distribution of PCBs reported by Li et al. [78] and shown in Fig. 4 is a typical global profile. Soil levels, however, are highly variable within countries and regions and influenced largely by the intensity of human activity (Fig. 3). Holoubek et al. [110], for example, examined 18 years of time trend POPs data collected in the southern Czech Republic since 1988 and reported significant variability in occurrence and distribution of selected groups of persistent pollutants in soil and sediment.

For comparison, the lowest ∑PCDD/PCDF concentrations reported worldwide in soil are from Jia et al. (2014) on the Fildes Peninsula, Antarctica, (0.015 pg I-TEQ/g), Arctic soil (0.33 pg I-TEQ/g), and Tibetan Plateau (0.37 pg I-TEQ/g).

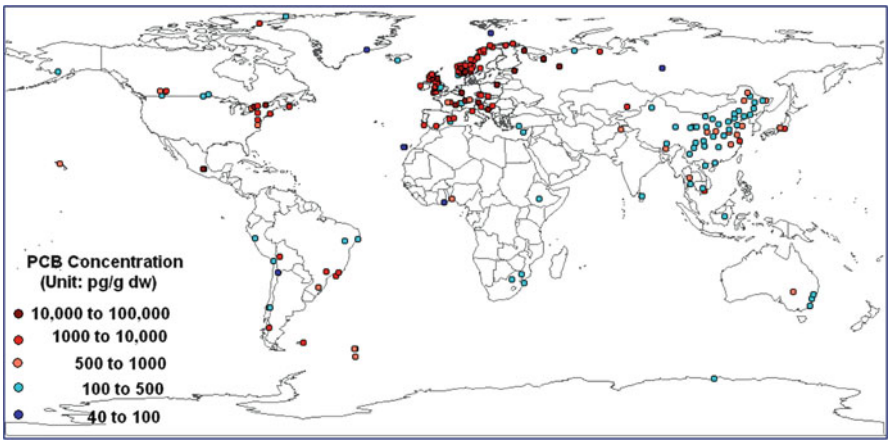

Fig. 4 Concentrations (pg/g dw) of ∑PCBs in soil around the world (adapted from [78])

These results are consistent with 1-year model simulations of atmospheric dioxin deposition reported by Booth et al. [111] and comparable to results of ambient air sampling conducted by Piazza et al. [112]. Studies conducted in high alpine regions such as the Italian Alps, Himalayas, Rocky Mountains, and Peruvian Andes monitoring several environmental compartments (air, soil, sediment, surface water, and foliage) simultaneously to understand the driving forces determining the distribution patters of POPs generally report similar observations [113].

Data describing POP levels in Africa are limited, but available data showing increasing levels of PCBs and other persistent contaminants can be related to growing industrialization and poor waste disposal practices [114]. The use of pesticides in Africa is likely a contributing factor as well [115, 116]. Studies on chlorinated pesticide occurrence in Botswana, for example, suggest that the residence time of OCPs in arid subtropics is very short. In the Okavango Delta [117], the low uptake capacity of the environment is likely due to the high temperatures and the low organic matter content of the soils. The few reported studies focus on a limited number of sampling sites for short periods of time; therefore, it is difficult to obtain information on the spatial variability of OCPs, identify their temporal trends, or gain an understanding of the fate of OCPs in low-latitude environments.

6 Levels in Aquatic Environments

While the relative importance of atmospheric and hydrospheric transport in controlling the global distribution of specific POPs remains uncertain, it is generally recognized that these two mechanisms are the predominant explanations for long-range environmental transport and global cycling of PCBs and most POPs. For ionic perfluorinated contaminants (PFCs) such as carboxylated and sulfonated

perfluoroalkyl acids, for example, negligible vapor pressure, water solubility, and moderate sorption to solids predict ionic PFC accumulation in surface waters; therefore, the environmental distribution of PFCs is likely governed by hydrodynamics [118]. For the dioxins, however, traditional physical processes involving transport of sediments and windblown particulate are widely accepted as the primary dispersive mechanisms.

The summary of PCDD/PCDF and PCB levels in sediments shown in Table 2 typifies the features of global distribution reported worldwide. Perhaps the largest influence of POPs on human health is their widespread occurrence in different marine and freshwater environments throughout the world [142]. Levels in freshwater environments tend to be higher than marine environments owing largely to oceans and estuaries behaving as sinks for coastal and inland sources. In addition to the legacy POPs, Loos et al. [143] found several emerging chemicals as among the chemicals of most concern in 100 European rivers from 27 European countries, where water samples tested positively for 35 pharmaceuticals, pesticides, PFOS, PFOA, benzotriazoles, synthetic and natural hormones, and endocrine disrupting chemicals.

China is believed to have some of the highest contamination levels in the aquatic environment because of rapid industrialization over the past 30–50 years. Surface water, sediment pore water, and sediments in the Pearl River Delta, Minjiang River estuary, Zhujiang River, Yellow River, and elsewhere both inland and in coastal areas have been shown to contain high levels of OCPs, PCBs, PBDEs, dioxins, and other POPs [144–147]. The spatial distribution characteristic of contamination in the whole country is generally believe to be southeast > central > northwest [148].

It is generally recognized that contamination is less evident in the Southern Hemisphere, though the situation may change in South America and Africa with further industrialization. Temporal trends for both PBDEs and HBCD in Asia are unclear currently, and e-waste recycling has raised concerns that this activity will lead to increased levels, particularly in Asia and Africa [149]. Klánová et al. (2008) reported soil concentrations ranged between 0.51 and 1.82 ng g^{-1} for seven indicator PCB congeners, between 0.49 and 1.34 ng g^{-1} for HCH congeners, between 0.51 and 3.68 ng g^{-1} for Σ DDT (i.e., *p,p*-DDT, DDE, and DDD), and between 34.9 and 171 ng g^{-1} for Σ16PAHs. Sediment levels from 0.32 to 0.83 ng g^{-1} were found for ΣPCBs, from 0.14 to 0.76 ng g^{-1} for HCHs, from 0.19 to 1.15 ng g^{-1} for ΣDDT, and from 1.4 to 205 ng g^{-1} for ΣPAHs. A prevalence of low-mass PAHs, less chlorinated PCBs, and more volatile chemicals indicates that the long-range atmospheric transport from populated areas of Africa, South America, and Australia is the most probable contamination source for the solid matrices in James Ross Island.

Recent studies in Chile and Colombia, for example, report levels of BFRs (up to 2.43 and 143 ng g^{-1} dw of PBDEs in Chile and Colombia, respectively) and UV-F (non-detect to 2.96 and 54.4 ng g^{-1} dw in Chile and Colombia, respectively) in the low range of published data from other regions of the world [167]. Miglioranza et al. [150], providing the first systemic data on levels of OCPs, PCBs, and PBDEs in soils, sediments, SPM, and surface water along the Rio Negro basin

Table 2 PCDD/PCDFs and dioxin-like PCBs (dl-PCBs) in surface sediment reported from around the world

Country/region	Sediment environment	Date	*N*	Σ PCDD/PCDFs (pg g^{-1} dw)	Σ dl-PCBs (pg g^{-1} dw)	Σ PCBs (pg g^{-1} dw)	PCDD/PCDF TEQ (pg TEQ/g)	PCDD/PCDF +dl-PCB TEQ (pg TEQ/g)	TEQ scheme	Reference
Africa										
South Africa (central region)	Freshwater	2006	7	42.8 (1.4–183)	0.20 (120–1,800)	–	0.26 (0.08–0.79)	0.46 (0.12–1.4)	WHO 2005	[119]
Morocco (Nador lagoon; Moulay Bousselham lagoon)	Marine	2006-2007	4	3,682 (960–3,683)	–	–	0.01 (0.004–0.04)	–	WHO 2005	[120]
Morocco (Ports of Tangier, Larache, and Kenitra)	Marine	2003-2007	4	75 (12.7–164)	–	–	0.86 (0.04–2.7)	–	WHO 2005	[120]
Kuwait (rural area)	Marine	NR	2	2.1* (0.4–3.8)	–	–	0.2	–	WHO 2005	[121]
Kuwait (industrial area)	Marine	NR	10	130.25* (12.5–131.5)	–	–	1.72 (0.2–4)	–	WHO 2005	[121]
Asia/Pacific										
Australia	Freshwater	2003	33	490* (ND–3,500)	160 (ND–1,300)	–	0.32 (0.0005–2.2)	0.41 (0.002–2.9)	WHO 1998	[50–52]
Australia	Marine	2003	12	460* (33–2500)	79 (0.018–440)	–	0.45 (ND–3.2)	0.53 (0.0000018–3.9)	WHO 1998	[50–52]
Australia	Estuarine	2003	30	14,000* (7.6–110,000)	3,200 (ND–28,000)	–	30 (0.0038–510)	32 (0.0038–520)	WHO 1998	[50–52]
China (Yellow River)	Freshwater	2010	13	2.1–19.8*	1.11–9.9	2.5 1.12–9.9	0.8–0.55	0.08–0.57	WHO 2005	[122]
China (Yangtze River)	Freshwater	2010	13	21.8* 6.1–84.9	1.8–24.1	1.79–27.07	0.09–0.47	0.13–0.29	WHO 2005	[122]

South Korea (Yellow Sea)	Marine	2008	12	35.3* (ND–142.1)	–	–	–	–	WHO 2005	[123]
China (Yellow Sea)	Marine	2008	28	10.35* (ND–62.4)	–	–	–	–	WHO 2005	[123]
China (Haihe River estuary and Dagu River)	Estuarine	2003	13	48,000* (151–557)	–	23,006 (775–1046)	20–975	21–996	WHO 2005	[124]
China (Kaifaqu and Yongdingxin River estuaries)	Estuarine	2003	2	291* (210–372)	–	1,045.5 (922–1,169)	1.7–2.7	1.8–2.8	WHO 2005	[124]
China (Changjiang River estuary)	Estuarine	2003–2004	10	170* (26–374)	–	–	0.8 (0.4–1.4)	–	WHO 2005	[125]
Hong Kong (Pearl River estuary)	Estuarine	NR	16	6,040* (4,439–9,404)	–	–	12.5 (10.8–16.4)	–	WHO 2005	Müller et al. (2002)
South Korea	Marine	1992	11	102–6493	–	–	1–76	–	WHO 1998	[65, 66]
South Korea (industrial bays)	Marine	2000-2002	122	216–755	–	–	1.2–7.2	1.3–10.8	WHO 2005	[126]
Japan, Toyano Lagoon	Estuarine	2000-2003	5	18,762 (370–54,000)	–	–	0.5–76.0	–	WHO 2005	[127]
Japan, Tokyo Bay	Marine	2000-2003	9	–	–	–	3.1–49	3.3–52.0	WHO 2005	[127]
Vietnam, coastal lagoons	Estuarine	2002, 2006	11	871* (197–2919)	–	–	1.85 (0.25–5.2)	–	WHO 2005	[128]
Vietnam, Saigon River estuary	Estuarine	2004-2005	12	650 (250–1800)	1,665 (18–8.400)	287–294	–	4.1 (0.73–17)	WHO 2005	[129]

(continued)

Table 2 (continued)

Country/region	Sediment environment	Date	*N*	Σ PCDD/PCDFs (pg g^{-1} dw)	Σ dl-PCBs (pg g^{-1} dw)	Σ PCBs (pg g^{-1} dw)	PCDD/ PCDF TEQ (pg TEQ/g)	PCDD/PCDF + dl-PCB TEQ (pg TEQ/g)	TEQ scheme	Reference
Europe										
Finland (Gulf of Finland)	Estuarine	1998-2003	44	15,300 (1,030–52,900)	–	–	83 (13–216)	–	WHO 2005	[130]
Italy (Venice Lagoon)	Estuarine	1996-1998	22	12.3* (1.9–34)	–	–	1.11 (0.28–2.15)	–	WHO 1998	[131]
Mediterranean Sea	Marine	2003-2004	6	413* (102–680)	–	–	3.7 (1.3–5.6)	–	WHO 1998	[132]
Portugal (Lima, Ria de Aveiro, Mondego, Tejo, Sado, Mira, and Ria Formosa estuaries)	Estuarine	2011	31	4.6–634.6*	–	16.0–11,278.8	0.1–5.3	0.1–11.6	WHO 2005	[133]
Spain (Catalonia coast)	Marine	2000	18 (PCDD/PCDF and dl-PCB) (45 (PCB)	1,790* (60–8,140)	7,750 (270–35,600)	53,500 (1,100–311,000)	8.88 (0.4–39.24)	12. 92 (0.43–42.76)	WHO 1998	[134]
Spain (Cantabria coast)	Marine	2006	6	1.7* (0.15–3.99)	396 (139–691)	2,493 (558–4,656)	0.1 (0.003–0.164)	0.3 (0.08–0.52)	WHO 1998	[135]
The UK (Tees, Thames, and Firth of Forth estuaries)	Estuarine	NR	35	–	–	–	0.41–18.3	3.42–18.3	WHO 1998	[136]

North America										
Trinidad, Port of Spain Harbor	Marine	2007	6	638 (403–1,157)	–	–	3.49 (2.16–6.27)	–	WHO 2005	[137]
The USA (Houston Ship Channel)	Marine	2002-2003	83	4,186* (2,737–6,502)	–	–	25.3 (17.6–32.6)	–	WHO 2005	[138]
The USA (Pensacola Bay)	Marine	NR	53	1,502* 19.8–60,825	838.1 (21.3–23,453)	–	4.6 (0.2–129.3)	6.7 (0.3–145.5)	WHO 2005	[139]
The USA (Detroit)	Freshwater	1998	35	348* (68.7–1,420)	(1,338–87,300)	–	20 (3–62)	22 (3–69)	WHO 1998	[140]
The USA (Florida panhandle)	Marine	2002	32	–	–	–	0.51–77.5	–	USEPA1989	[141]

Data presented as mean (range). The Σ PCDD/PCDFs data represent the mean and range for reported measurements of all dioxin and furan congeners; an asterisk (*) indicates the mean and range represent the sum of the seventeen 2378-substituted congeners. NR indicates the sampling date was not reported by the authors

in Patagonia Argentina, reported levels spatially distributed consistent with historical intense pesticide application, the urban and industrial discharges, and the presence of hydropower dams. Verhaert et al. [151] reported similar results in the Congo River basin, where, in general, levels of PCBs, PBDEs, and OCPs in different environmental compartments were low compared to other studies around the world.

Understanding POP levels in surface water is far more unpredictable and uncertain than in soil and sediment because of dynamic and ever-changing source inputs, suspended organic matter levels, and climatic conditions that typify the hydrosphere. Periodic monitoring of PCDD/PCDFs in sediments over several years in German rivers and in San Francisco Bay, California, highlights how environmental conditions can improve or degrade quickly and almost randomly (Table 3). Similar observations are often noted for other POPs in surface waters; historical sampling results provide, at best, a temporal snapshot of regional conditions that may not be representative of current conditions.

Furthermore, debate continues on surface water sampling methodologies. Menzies et al. [154], for example, has called attention to sampling methodology as possible reason for differences in marine surface water levels reported around the world, suggesting that even in so-called "surface" samples, the differences may be attributable, at least in part, as much to sampling protocol as to proximity to sources and long-range transport mechanisms. Sampling the sea water microlayer comprising the top few centimeters of the water column, Menzies et al. [154] found six different categories of POPs, chlorobenzenes, hexachlorocyclohexanes, chlordane-related compounds, organochlorine pesticides and other cyclodiene pesticides, DDT and metabolites, and polychlorinated biphenyls, in sea water collected between 1997 and 2001 near shore coastal marine locations and oceanic islands, atolls and reefs in the western Caribbean, Pacific coasts of Central and South America, and the tropical South Pacific. The concentrations observed were generally low compared to other regions in the northern hemisphere, ranging from <1 ng L^{-1} to 18.45 ng L^{-1}. Other studies also report higher concentrations of organic compounds by focusing on the sea-surface slick as compared to more traditional water sampling reported in other studies conducted in the same region [155–157]. Comparing sea-surface microlayer (SML) and seawater samples collected from Singapore's coastal marine environment and analyzed for selected chlorinated pesticides and PCBs, Wurl and Obbard [157] reported that concentration ranges of ∑HCH, ∑DDT, and ∑PCB in subsurface (1 m depth) seawater were 0.4–27 (mean 4.0), 0.01–0.6 (mean 0.1), and 0.05–1.8 ng L^{-1} (mean 0.5 ng L^{-1}), respectively. In the SML, the concentration ranges of ∑HCH, ∑DDT, and ∑PCB were 0.6–65 (mean 9.9), 0.01–0.7 (mean 0.2), and 0.07–12 ng L^{-1} (mean 1.3 ng L^{-1}), respectively.

Regional differences may also play a role in shaping global trends. According to Verhaert et al. [151], some researchers have suggested that the environmental fate of POPs in tropical ecosystems is different from temperate and cold ecosystems, because of the prevailing high temperatures and heavy rainfall [158] and higher leaching and volatilization [159]. Some suggest that tropical regions also may act as a sink since removal processes (microbial transformation and chemical hydrolysis)

Table 3 Periodic monitoring of PCDD/PCDF concentrations (pg I-TEQ/g) in surface waters in Germany and California, USA

Location	Date	Range or maximum level	Reference
Germany			
Elbe River, Elbemeßstationen	1990	36–167	[152]
Elbe River, Elbe-Messstationen	1994	339	
Elbe River, Hamburger Hafen	1992	1,500	
Elbe River, Hamburger Hafen	2001	113	
Spittelwasser	1992	1,500	
Spittelwasser	2001	83,000	
Saale/GÜSA-Messstellen	1994	57,6	
Bode/Unterlauf ab Staßfurt	1995	1,170	
California, USA (pg TEQ/L)			
Sacramento River	January 2002	0.029	[153]
	July 2002	0.048	
	January 2003	0.025	
	August 2003	0.032	
Yerba Buena Island	January 2002	0.046	
	July 2002	0.071	
	January 2003	0.026	
	August 2003	0.057	
Dumbarton Bridge	January 2002	0.259	
	July 2002	0.073	
	January 2003	0.079	
	August 2003	0.041	

The Σ PCDD/PCDFs data are reported using WHO 1998 TEFs for the sum of 17,2378-substituted congeners

may be faster compared to temperate and Arctic regions [160–163]. Recent studies indicate POPs are mobile in the tropical rainforest soils due to fast litter turnover (leading to rapid POP transfer to the subsoil) and leaching rates exceeding degradation rates especially for hydrophobic substances; these results suggest higher overall storage capacity of tropic soils in comparison to colder environments [164].

7 Summary and Conclusions

Our scientific knowledge and understanding of environmental levels have improved considerably since the 1990s. Lohmann et al. [165] point to several challenges that have not yet been overcome appreciably to the present time. Scientists would agree with the need for a "global mass balance," which combines knowledge of source emission rates with the quantification of environmental reservoirs and final sink fluxes. However, accurate emission rates remain a challenge for legacy and emerging POPs. To date, it continues to be much easier to account for inventories of POPs

(e.g., their presence in soils or sediments), but we have more difficulties in estimating historical emissions, removal fluxes through reactions, and the presence of POPs in remote locations, such as deep oceans and the polar regions.

Nizzetto et al. [166] also point to knowledge gaps and the need for better understanding of past, current, and future trends of POPs in the environment, which requires accounting for both primary emissions and reemissions to the atmosphere from reservoirs in the global environment. These reservoirs, which include soils, vegetation, biota, water bodies, and sediments, confound our understanding of environmental trends and global cycling. Improvements in sampling and analytical methodologies, monitoring program, and environmental models will continue to close the knowledge gap and improve our understanding of how human activities impact our environment. Dr. Hutzinger was at the forefront of this effort throughout his scientific career. It is left to future generations of scientists to follow in his footsteps and travel beyond.

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Levels, Trends, and Health Effects of Dioxins and Related Compounds in Aquatic Biota

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Abstract The objective of this chapter is to review current knowledge of the levels, trends, and health effects of dioxins and dioxin-like compounds (DLCs) including polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs), and polychlorinated biphenyls (PCBs) in aquatic biota, with a special focus on high trophic level species. DLCs can be released into the environment through storm runoff, air deposition, wastewater discharge from industrial processes, and leaching from landfills. To characterize their influences in biota, studies examining levels and trends of DLCs from invertebrates to vertebrates from several regions (the Arctic, North America, Asia and Europe) are reviewed. Over several decades, such studies have helped elucidate the accumulation, possible sources, metabolic fate, as well as the potential health effects of dioxins and DLCs in aquatic biota. The trophic transfer of these compounds via bioaccumulation and biomagnification can result in higher concentrations in top predators, and a wide range of toxic effects (e.g., endocrine disruption, developmental and reproductive effects, and immunotoxicity) has been reported in diverse species, especially those occupying high trophic levels, e.g., marine mammals. Because of their high trophic position and widespread distribution, marine mammals are valuable sentinel species for PCB and DLC contamination, providing insights into possible sources, transport pathways, and the distribution of these compounds on a global scale. Population-levels effects related to contaminant-induced reproductive impairment and disease have been reported in wildlife inhabiting polluted regions, and the occurrence of mass mortalities among marine mammal populations has been linked to high body burdens of immunotoxic compounds, notably PCBs. Many affected populations have never recovered to their original levels. For many contaminant-stressed populations, the added stress of climate change is exacerbating the problem, causing shifts in food webs and increasing both the distribution and toxicity of POPs in coastal and oceanic environments. Critical data gaps and future research challenges are highlighted as areas that require further study.

Keywords Aquatic biota, Brominated dibenzo-*p*-dioxins and dibenzofurans, Chlorinated dibenzo-*p*-dioxins and dibenzofurans, Climate change, Dioxin-like PCBs, Immunotoxicity, Marine mammals, Mass mortalities, Polychlorinated biphenyls, Trophic transfer

Contents

1 Introduction

The environmental and human health implications of dioxins and dioxin-like compounds (DLCs) have been of ongoing concern since the mid-1980s. "Dioxins" generally refers to 75 congeners of polychlorinated dibenzo-*p*-dioxins (PCDDs) and 135 congeners of dibenzofurans (PCDFs). Seventeen of these 210 PCDD/Fs congeners have chlorine atoms in the 2, 3, 7, and 8 positions of the molecular structure and exhibit high toxicity [1]. Twelve of the 209 polychlorinated biphenyl (PCBs) congeners, with a similar stereostructure to PCDD/Fs, i.e., PCB-77, -81, -105, -114, -118, -123, -126, -156, -157, -167, -169, and -189, are known as dioxin-like PCBs (dl-PCBs) or DLCs. These 29 compounds can be bound to the aryl hydrocarbon receptor (AhR) and induce the AhR-mediated toxic responses. They also were among the first group of compounds to be included in the toxic equivalency factors (TEFs) scheme by the World Health Organization (WHO) in 1997 [1]. The most toxic congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is used as a reference congener in the TEF scheme with a TEF value defined as 1, and the toxicity of other dioxins and DLC was assigned a value in between 0 and 1 relative to 2,3,7,8-TCDD's TEF. The concepts of TEFs and toxic equivalent (TEQ) concentrations have been introduced to facilitate risk assessments and the regulatory control of dioxin and DLC exposure [1, 2]. Later on, in 2011, a joint WHO and United Nations Environment Programme (UNEP) expert panel evaluated and extended the TEF scheme of dioxin-like compounds to include polybrominated dibenzo-*p*-dioxins (PBDDs), polybrominated dibenzofurans (PBDFs), and certain dioxin-like polybrominated biphenyls (PBBs) [3]. A detailed review of the validity and criteria for inclusion in the TEF concept was recently completed [2, 3]. Although there are a large number of other halogenated compounds (e.g., polychloronaphthalenes, polybrominated naphthalenes, mixed halogenated dibenzo-*p*-dioxins, mixed halogenated dibenzofurans, hexachlorobenzene, and polychloroterphenyls, among others) which meet the inclusion criteria for the TEF scheme, they are not included herein due to the lack of environmental and toxicological data. The chemical structures of PCDD/Fs, PBDD/Fs, PCBs, and related compounds are shown in Fig. 1.

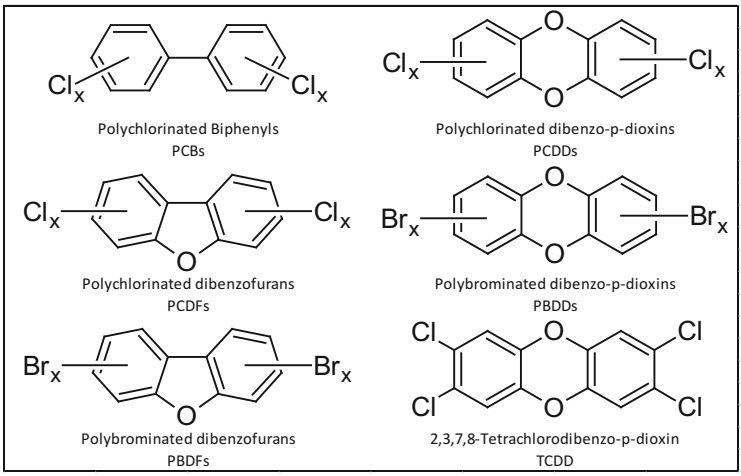

Fig. 1 Structures of dioxin and dioxin-like halogenated compounds

PCDDs and PCDFs are released into the environment from different combustion processes [4–7], and are sometimes present in various chlorinated chemical formulations as byproducts or impurities [8–10]. These compounds can be produced by a wide range of manufacturing processes such as incineration and iron ore sinter plants, as well as from accidental fires. Among the different emission sources, higher levels can be produced by waste treatment processes that use incinerators without proper emission controls [11, 12]. PBDDs and PBDFs have been found in combustion gases (processes) and combustion engines [13–15], and may occur as impurities in commercial PBDE (polybrominated diphenyl ether) mixtures [16, 17]. Large amounts of PBDD/Fs may be formed during fires under uncontrolled combustion conditions in the presence of PBDEs [15, 18–20]. In contrast to the unintended generation of dioxins, PCBs were manufactured for industrial usage and they were typically released from the application or disposal of industrial PCB-containing products. Although the domestic production of PCBs was banned in 1979, PCBs are still used in closed systems such as transformers, capacitors, and other electrical equipment. Four decades later, PCBs can still be detected in the air, water, sediments, soil, and biota. Most PCBs in water are bound to particulates and sediments, and are slowly released over a long period of time (decades). Because of their stability and ongoing inputs, PCBs are subject to recycling in food webs and will remain an environmental concern for the foreseeable future [21].

Because of their lipophilic nature and resistance to metabolism, PCBs, dioxins, and related compounds bioaccumulate and biomagnify in the fatty tissues of animals and humans. PCBs can easily reach higher concentrations in predator species at the upper trophic levels [22, 23] and are the predominant contaminants in most wildlife populations today [24, 25]. High levels of dioxins and PCBs have been detected in dolphins [26–28], harbor seals (*Phoca vitulina*) [25, 29, 30],

northern fur seals (*Callorhinus ursinus*) [31], hooded seals (*Cystophora cristata*) [32], Steller sea lions (*Eumetopias jubatus*) [33], sharks [27, 34], predatory birds [28, 35], and human tissues [28, 36–38]. Toxic responses to DLCs including immunotoxicity, endocrine disruption, and adverse effects on reproduction and development have been observed in diverse species of biota [39–42]. A large amount of data suggests that PCBs and DLCs have adversely affected the health of marine mammals [40, 41, 43–45], and are associated with endocrine-disrupting effects, immune suppression, infertility, skeletal abnormalities, and population declines among seals from the North and Baltic Seas [44, 46–49]. PCBs are also associated with a high prevalence of neoplasms and carcinoma that cause mortality in California sea lions (*Zalophus californianus*) [50]. Reproductive effects resulting in declines in otter (*Lutra lutra*) populations in Europe have been linked to high concentrations of OC contaminants, notably PCBs [44, 51–53].

PCBs and PCDD/Fs have long been suspected to play a role in the recurring epizootics and large-scale mortalities among marine mammals, including US Atlantic coast harbor seals [54, 55] and bottlenose dolphins (*Tursiops truncatus*) [56]; seals in the Baltic and North Seas [57, 58]; Mediterranean striped dolphins (*Stenella coeruleoalba*) [59–61]; Baikal seals (*Phoca sibirica*) [62]; and Caspian seals (*Phoca caspica*) [63], among others. Feeding studies conducted in the mid-1980s and 1990s demonstrated the sensitivity of harbor seals to PCB and PCDD/F exposure and helped establish a threshold level for PCB-related toxic effects in adult marine mammals. Harbor seals fed PCDD/F and PCB-contaminated fish exhibited reproductive impairment [48], reduced plasma thyroid hormone and retinol levels [64], and the suppression of numerous cellular and humoral immune functions [65, 66]. Recent biomarker studies indicate that nursing seal pups with relatively low blubber concentrations of PCBs may be vulnerable to immune- and endocrine disrupting effects during a sensitive window of development [25, 67–70].

The study of temporal trends for PCBs, dioxins, and other DLCs in biota is useful to aid policy makers in developing effective regulatory policies for the protection of public health and the environment. Trend studies provide information that places current environmental inputs and chemical loading into context and helps in the assessment of potential future impacts. This chapter provides an overview of levels, trends, and health effects of PCDD/Fs, PBDD/Fs, and PCBs in aquatic species from invertebrates to vertebrates from different regions, including phytoplankton, zooplankton, shellfish, fish, turtles, seabirds, and marine mammals. Knowledge gaps and potential future research perspectives are highlighted.

2 Sources

2.1 PCDD/Fs and PBDD/Fs

PCDD/Fs and PBDD/Fs are the byproducts of many industrial and combustion processes. Social and scientific concerns regarding PCDD/Fs increased with the detection of TCDD in Agent Orange [71], the herbicide that was widely used during the Vietnam War [71, 72]. Three mechanisms for the formation of PCDD/Fs during incineration have been proposed. These mechanisms include the input of wastes containing unburned PCDD/Fs, combustion processes in the presence of precursors (chlorinated compounds) of PCDD/Fs, and de novo synthesis [73–77]. In accordance with the Stockholm Convention, the USA, Canada, Australia, Japan, and certain European countries have performed nationwide surveys to create an emission inventory of PCDD/Fs in the atmosphere from 1993 to 1997, and these data were intended to identify the potential emission sources of PCDD/Fs [78]. The major emission source of PCDD/F is waste incineration, including municipal, hazardous, and industrial types, which accounted for 69% of the total PCDD/F emissions to the atmosphere. The next highest contributors to the total emissions of PCDD/Fs are the combustion process of iron and steel (10%) and non-ferrous metal processing (8%). Other emission sources of PCDD/Fs are chloralkali processes, the bleaching of pulp and paper, the burning of chlorine–containing fuels, and the production of pentachlorophenols [78–85].

PBDD/Fs are also unintentional by-products of incineration processes, and they have similar physico-chemical properties and anthropogenic sources as PCDD/Fs [86]. An important source of PBDD/F is the incineration of products containing BFRs and the thermolysis of BFR material, e.g., PBDEs during fire events [18–20]. PBDD/Fs can also be formed during thermal processing procedures of PBDEs such as polyeurethane foam extrusion, molding and recycling, and degradation [19, 86, 87] and ultra-violet irradiation of deca-BDE [88]. Additionally, PBDD/Fs are found at trace levels as impurities in commercial BFR products, such as DE-71, DE-79, DE-83, and some deca-BDE products [16, 17, 89].

2.2 PCBs

From 1929 until their prohibition in 1979, PCBs were in widespread use as closed systems and heat transfer fluids, hydraulic fluids and lubricants, plasticizers, and fire retardants [9]. Over five decades, the USA was responsible for approximately half of the world's production of PCBs and imported 50% of the PCBs produced by other countries [90]. PCBs are still authorized for use in many applications [9]. Fresh sources of PCBs include in-service electrical equipment, transformers, machinery, manufacturing sites, building materials, landfills and scrap yards, and waste and recycling operations, many of which are located in densely populated

urban/industrial centers [9, 91]. Because of evidence of adverse health effects in wildlife and humans, PCB production and use was banned by most developed countries in the late 1970s. However, approximately 1.5 million metric tons were produced worldwide in addition to 650,000 metric tons in the USA, and large volumes of PCBs are still contained in transformer equipment and landfills [92]. Incomplete combustion and industrial processes are also an important source of PCBs [93, 94]. Emerging evidence suggests that non-legacy PCBs are unintentionally present in pigments used for dyes, inks, and paints [95]. It was estimated by the late 1980s that only about 1% of all PCBs had reached the oceans, while about 30% had accumulated in dumpsites and sediments of rivers, coastal zones, and estuaries [96]. Because of fresh inputs (from current permitted uses) and vast environmental reservoirs, PCBs are expected to remain the predominant contaminants in aquatic and marine biota at least until 2050 [21].

3 Levels and Trends in Aquatic Biota

3.1 Invertebrates

Understanding the accumulation of dioxins and DLCs in phytoplankton is essential for assessing the occurrence, transport, and distribution of these contaminants in aquatic environments. Phytoplankton uptake influences the fate and transport of pollutants since it is a key step in the transfer of pollutants from water to fish [97–99]. In addition, phytoplankton uptake and the subsequent transfer to zooplankton result in depositional fluxes of organic pollutants in underlying aquatic environments [100].

Joiris and Overloop [101] determined the concentrations of PCBs in phytoplankton and zooplankton that were collected from the Indian sector of the Southern Ocean in 1987. The PCB concentration in netplankton (200 μm mesh size, primarily zooplankton) was 0.35 μg/g dry weight (dw) or 5.8 μg/g lw, which was about half of that measured in phytoplankton (0.74 μg/g dw) on a dry weight basis and about a third of the phytoplankton level (16.3 μg/g lw) on a lipid weight basis. The difference between phytoplankton and zooplankton was even more extreme when reported on a water volume basis thereby emphasizing the need for consistent units when comparing studies. The PCB levels in this study were similar to those found in North Sea zooplankton (0.7 μg/g dw) [102]. Galbán-Malagón et al. [103] recently reported the concentrations of PCBs and other organochlorines in seawater and phytoplankton from the Southern Ocean during their Antarctic cruises in 2005, 2008, and 2009. The PCB concentrations in phytoplankton from this study ranged from 0.0027 to 0.014 μg/g dw which was 1–2 orders of magnitude lower than the 1987 study. The long-term decreasing trends in PCB levels were also found in seawater from the Southern Ocean, with a half-life of 5.7 years.

Seawater and planktonic copepods (*Calanus glacialis* and *C. hyperboreus*) were collected from the Alaskan and Canadian Arctic regions to investigate the spatial distribution and bioaccumulation of organochlorines (OCs) such as PCBs [104]. The PCB concentrations in Alaskan and western Canadian zooplankton (12.6–33.8 ng/g dw) were comparable to those from northern Baffin Bay (30.2 ng/g dw) but lower than those from Rankin Inlet in central Canada (54.5 ng/g dw).

Okumura et al. [105] reported the bioaccumulation of PCDD/Fs and dl-PCBs in lower trophic level organisms collected from Sendai Bay, Japan. The total concentrations of PCDDs, PCDFs, and dl-PCBs in phytoplankton were 150, 12, and 51 pg/g wet weight (ww), respectively. The PCDD/F concentrations in zooplankton (which are primary consumers) were lower than the levels in phytoplankton (a primary producer), but the dl-PCBs concentrations in zooplankton were higher than the levels in phytoplankton thereby indicating a difference in bioaccumulation between PCDD/Fs and dl-PCBs at the lowest trophic levels.

Wan et al. [106] reported PCDD/Fs and PCBs in a marine food web including the phytoplankton and zooplankton collected from Bohai Bay, China. The concentrations of PCDD/Fs were 28.9 pg/g ww (0.50 pg TEQ/g ww) in phytoplankton and 18 pg/g ww (0.32 pg TEQ/g ww) in zooplankton. Dl-PCB concentrations were 57.8 and 8.4 pg/g ww (0.50 and 0.32 pg TEQ/g ww) in phytoplankton and zooplankton, respectively.

Peltonen et al. [107] reported the concentrations of PCDDs, PCDFs, and PCBs in offshore zooplankton (size from 0.2 to 20 mm) collected in 2001, 2002, and 2010 from the northern and central Baltic Sea. Concentrations of PCDD/Fs were 10.1–21.7 pg/g lw WHO_{2005}-TEQ in 2001/2002 and were 7.2–19.3 pg/g lw WHO_{2005}-TEQ in 2010. Among the PCDD/Fs, the most toxic congeners 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, and 2,3,4,7,8-PeCDF contributed above 80% of the total toxicity of PCDD/Fs. PCB concentrations were 6.0–12.9 pg/g lw WHO_{2005}-TEQ in 2001/2002 and were 3.7–9.1 pg/g lw WHO_{2005}-TEQ in 2010 and few temporal differences were noticed. However, 1,2,3,4,6,7,8-HpCDF and OCDF were found to predominate in 2001–2002, especially in the eastern Gulf of Finland (average concentrations 50 and 89 pg/g lw, respectively). The PCB concentrations were highest in the Gulf of Finland and in the Bothnian Bay, and concentrations of most PCBs were somewhat higher in 2001–2002 than in 2010. Among the dioxin-like PCBs, the concentrations of PCB-77 were highest (271–572 pg/g lw), followed by PCB-126 (32–113 pg/g lw), PCB-169 (5.81–25.5 pg/g lw).

Dynamic bioaccumulation models for these contaminants have been developed by researchers through field surveys of dioxins and DLCs in plankton [98, 108]. Dachs et al. [108] developed dynamic models that couple air–water exchange and the phytoplankton uptake of POPs and then applied them to field PCB measurements. The simulation results suggested that air–water exchange and other atmospheric-derived inputs primarily contribute to the POP concentrations in phytoplankton. In addition, this process suggested a major role in the global cycling

of POPs, with POP atmospheric deposition considered a major contamination source for the world's oceans.

Shellfish are useful sentinel species for studying levels and trends of contaminants in aquatic environments. Diaz [109] measured dioxins in shellfish from the Oakland Bay in Washington State, USA and found that the dioxin concentrations in Manila clams ranged from 0.05 to 0.27 pg/g lw, which were similar to those in Pacific oysters (0.13–0.37 pg/g lw), and lower than those in Kumamoto oysters (0.3–0.6 pg/g lw). In mussels, total dioxin concentration was about 0.17 pg/g lw. Parera et al. [110] analyzed PCDDs, PCDFs, and PCBs in marine shellfish (murex, carpet shell, and mussel) of the Ebro River Delta, Spain and found that PCDD/F concentrations were 0.29–1.17 pg/g ww, and the concentrations of dl-PCBs were 24.6–399 pg/g ww. PCDFs contributed a larger content to WHO-TEQ_{2005} extent than PCDDs. A slight decrease of PCDD/F and dl-PCB concentrations was noticed from 2006 to 2012, which was in agreement with the decrease in PCDD/F and PCB concentrations observed in human serum in Spain [111]. The levels of PCDDs, PCDFs, and PCBs for 123 Spanish commercial oyster, mussel, and clam samples from 1995 to 2003 were determined and there was a significant decrease of dioxin and dl-PCB concentrations since 1995. The decrease of dioxin levels was more obvious than that of dl-PCBs, especially during the early years of the study [112]. Munschy et al. [113] evaluated the levels and temporal trends of PCDD/Fs in archived marine mussels collected between 1981 and 2005 from selected sites along French coasts and noticed a pronounced decrease in PCDD/F concentrations over the 24-year period at most sites, except Toulon on the Mediterranean Sea.

In 2005, PBDDs were identified and reported in blue mussels from the Baltic Sea [114], and the concentration of total triBDD was estimated to be 170 ng/g lw [114]. Haglund et al. [115] measured PBDDs in marine fish, mussels, and shellfish from the Bothnian Bay and Bothnian Sea, the West Coast of Sweden, and the Baltic Proper. They found that the levels of PBDDs were higher in mussels than in other species, and there was an increasing temporal trend of PBDDs in mussels with an average annual increase of 11% from 1995 to 2003 [115]. Mussels, oysters, and scallops in Scotland were analyzed for PCDD/Fs, PBDD/Fs, PCBs, and other compounds [116]. PBDFs predominated over brominated dioxins. Generally, mussels and oysters had relatively higher levels of contamination than scallops, and their levels of contamination in the Southern beaches were greater than those in the north and northwest, which was consistent with Scottish industrial activity levels [116]. Fernandes et al. [117] also investigated PBDD/Fs in Pacific oysters (*Crassostrea gigas*), native oysters (*Ostrea edulis*), mussels (*Mytilus edulis*), scallops (*Pecten maximus*), and cockles (*Cerastoderma edule*) collected between 2006 and 2007 in the UK. PBDFs were detected more frequently and generally at a higher level than PBDDs. Oysters and mussels displayed relatively higher levels of PBDD/Fs. The levels of PBDD/Fs were consistent with the extent of local industrialization with lower levels observed in more remote areas such as the north of Scotland [117].

3.2 Fish

Monitoring environmental levels and trends of contaminants in fish is useful for studies of contamination levels and patterns in aquatic ecosystems, and for assessing potential health risks associated with wildlife and human consumption [118]. Kiviranta et al. [119] analyzed Baltic herring samples caught from the Baltic Sea during the spring periods of 1993–1994 and 1999 for PCDD/Fs and PCBs. Concentrations of some PCDD/F congeners and some PCB congeners in herring measured in 1993–1994 in the Gulf of Finland showed a clear correlation with the age of herring, which is consistent with the bioaccumulation of PCDD/Fs and PCBs. The PCDD/F concentrations ranged from 1 to 27 pg TEQ/g ww, and PCB concentrations reached 32 pg TEQ/g ww. No clear downward concentration trend of PCDD/Fs and PCBs in herring was observed between 1993 and 1994 and 1999. Karl et al. [120] determined PCDD/F and dl-PCB levels in the muscle of herring collected in 2006 and compared them with their previous study conducted at the same location in 1999 from the Western and Central Baltic Sea. The results from the 2006 study showed that PCDD/Fs and dl-PCBs of all herring samples were found to be below the maximum and action levels, and the average concentration was 3.55 pg TEQ/g ww. The comparison between 2006 and 1999 did not reveal obvious change in contamination levels during the 7-year time period. Similarly, PCDD/F and dl-PCB concentrations in Baltic herring (*Clupea harengus*) from the Swedish coast were found to be relatively stable since the mid to late 1990s; however, a general decreasing trend was seen for TEQ_{2005} PCDD, PCDF, and dl-PCB values at all sites, especially in the southern Bothnian Sea since 2001 [121]. It is unknown why concentrations in Baltic herring are not following the decreasing trend observed in other environmental matrices [121]. In Finland, altogether 344 samples of Baltic herring from 1978 to 2009 were collected across the Finnish coast of the Baltic Sea [122]. During the 31-year period, PCDD/F and PCB concentrations decreased about 80%, from 20 to 5 pg TEQ/g ww. The current concentrations of PCDD/Fs and PCBs in Baltic herring are relatively low, and mostly below EU maximum accepted levels, and are expected to continue decreasing [122].

Haglund et al. [115] determined PBDDs in marine fish from the Bothnian Bay and Bothnian Sea, the West Coast of Sweden, and the Baltic Proper. The levels of PBDDs in littoral fish generally exceeded those of PCDDs in Baltic Proper. Recently, Haglund et al. [123] reported the PBDDs in perch (*Perca fluviatilis*) from a Baltic Sea background contaminated area between 1990 and 2005. Although no temporal trend was found, large variations of PBDD concentrations were observed between consecutive years. Ashizuka et al. [124] measured PBDD/Fs in fish samples from three regions in Japan. 1,2,3,4,6,7,8-HpBDF was the most abundant congener of PBDFs with concentrations of 0.10–25.6 pg/g ww.

Bordajandi et al. [125] determined PCDD/Fs and PCBs in edible fish, namely wedge sole (*Dicologoglossa cuneata*), common sole (*Solea vulgaris*), white seabream (*Diplodus sargus*), sardine (*Sardina pilchardus*), and angler fish (*Lophius*

piscatorius) from the Coast of Huelva, on the Spanish southwest Atlantic coast. Total PCB concentrations were 861–23,787 pg/g ww, while 2,3,7,8-PCDD/Fs concentrations ranged from 0.2 to 1.18 pg/g ww. PCDD/F concentrations ranged from 0.038 to 0.186 pg TEQ/g ww, values well below the maximum concentrations established by the EU. PCBs contributed most to the total TEQ content in most species studied. Gómara et al. [112] also investigated PCDD/F and PCB content of 123 Spanish commercial salmon, tuna fish, and sardine samples from 1995 to 2003. A significant decrease in dioxin and non-ortho PCB concentrations was found over the years. The decrease was greater for dioxins than for non-ortho PCBs, especially during the early years of the study. The high contribution of PCBs to total WHO-TEQs in the fish species investigated suggests that it is important to monitor PCBs in fish products, and they should be included in further research and future legislation [112]. Parera et al. [110] reported the concentration trends of PCDD/F and dl-PCBs during 2006–2012 in marine fish in the Ebro River Delta area (Spain) and found that the concentrations of PCDD/Fs and dl-PCBs ranged from 0.03 to 0.31 pg TEQ/g ww and from 0.02 to 3.15 pg TEQ/g ww, respectively. All levels were below the maximum concentrations established by the EU Regulation. A slight decreasing trend in the levels of PCDD/F and dl-PCBs in fish was found from 2006 to 2012.

Hickey et al. [126] updated earlier reports with data from 1991 to 1998 for lake trout (*Salvelinus namaycush*) (Lake Erie only) and walleye (*Sander vitreus*) from the Great Lakes and quantified contaminant trends using multi-compartment models. As found in the past, fish from Lakes Michigan, Ontario, and Huron had the highest levels of PCBs. In the period after curtailment of chemical use, concentrations rapidly decreased, due to their relatively short half-lives from approximately 1 to 9 years. For dioxin-like PCBs, levels have not been decreasing during the 5-year period (1994 to 1998) [126]. Bhavsar et al. [127] measured concentrations of the seventeen 2,3,7,8-PCDD/Fs in lake trout (*S. namaycush*) or lake whitefish (*Coregonus clupeaformis*) collected in 1989–2003 from the Canadian Great Lakes. 2,3,7,8-TCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDD, 1,2,3,7,8-PeCDF, and 2,3,4,7,8-PeCDF were the most dominant congeners. The highest TEQs were from Lake Ontario lake trout (22–54 pg/g) while the TEQs for the other Canadian Great Lakes were 60–95% lower. A linearly decreasing trend for PCDD/Fs in lake trout from Lakes Ontario and Huron was found. There was no monotonously increasing or decreasing trend found for Lake Superior lake trout.

Brown et al. [128] measured PCDD/Fs and dl-PCBs in fish collected from San Francisco Bay in 2000 and from the California coast in 2001. The samples were composites of only the edible portions of the fish (skin on, skin off, or whole body minus head and guts) of comparable size and from distinct geographical areas. For all fish of all species, the mean PCDD/F was 33.1 pg TEQ/g lw. The mean for PCB-77, -126, and -169 was 109 pg TEQ/g lw. The highest concentrations of PCDD/F and dl-PCBs were found in the highly populated areas of San Francisco Bay, the Los Angeles area, and San Diego Bay.

3.3 Turtles

Snapping turtles (*Chelydra serpentina*) have been commonly used to evaluate the extent of organic chemical contamination and trends in the Great Lakes [129–139] and the Hudson River of New York [140, 141] since the 1970s. Snapping turtle eggs are also excellent bioindicators of the health conditions in wetlands and the bioavailability of organic contaminants. Snapping turtle eggs provide comprehensive information concerning the temporal and spatial trends of PCDD/Fs and PCBs [134, 136, 139]. Bonin et al. [142] compared the OCP and PCB levels in 39 snapping turtle clutches collected from ten sites along a highly polluted stretch of the St. Lawrence River in Canada. The results showed a high inter-site variability in PCB concentrations, which was consistent with those found by Bishop et al. [130] and Struger et al. [139]. De Solla et al. [135] reported a significant correlation between PCB concentrations in snapping turtle eggs and the industrial use of specific technical PCB mixtures in these areas. Hong et al. [143] reported the occurrence of planar, mono- and di-ortho PCBs in the fat tissues of snapping turtles that were collected in 1988 from rivers in the USA. The highest concentrations of PCBs (1,010 μg/g ww) and TEQs (106,000 pg TEQ/g ww) were found in snapping turtles from the Grasse River in northern New York, where extensive PCB and PCDF contamination was associated with the aluminum industry. Relatively higher PCBs (258 μg/g ww) and TEQs (47,000 pg TEQ/g ww) were also found in snapping turtles from the Snye Marsh of the St. Lawrence River, which is contaminated by large point sources. Dabrowska et al. [132] measured the TEQ concentrations of dl-PCBs in the fat tissue, eggs, and plasma of snapping turtles from the Ohio Basin of Lake Erie, USA. The TEQ concentrations, which were based on mammal-specific TEFs, ranged from 1.4 to 6.9 pg/g ww and from 21 to 582 pg/g ww in plasma and eggs, respectively. Significant correlations were found for PCB concentrations among fat tissues, eggs, and plasma from snapping turtles.

In comparison with PCBs, few studies are available on the PCDD/F concentrations in snapping turtle eggs or tissues. Most studies have reported the predominance of 2,3,7,8-TCDD for PCDDs and 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF for PCDFs in snapping turtle eggs, depending on various factors such as the metabolism and the specific source of these contaminants [129, 134, 139]. Similar to the occurrence of PCDD/F congeners in eggs, the fat and liver tissues of snapping turtles from the St. Lawrence River, USA, were also dominated by 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF [137].

Sea turtles are relatively sedentary, long-lived reptiles containing large fat bodies for POP accumulation [143–146]. Although known to be robust to physical damage, sea turtles are surprisingly very susceptible to chemical contaminants [147]. Following a long (10-year) open-water pelagic developmental phase, juvenile turtles settle in near-shore environments and forage on seagrass and algae close to land-based contaminant sources. All seven species of marine turtles worldwide are currently threatened or endangered, thus, sea turtles are important bioindicators of the levels and trends of chemical contamination in near-shore marine biota

[145, 146, 148–152]. Keller et al. [145] investigated PCBs and OCPs and their possible health effects on loggerhead sea turtles (*Caretta caretta*) from Core Sound, North Carolina by associating their concentrations with clinical health assessment data, including hematology, plasma chemistry, and body condition. They found that PCBs and other OC contaminants might affect the health of loggerhead sea turtles even though sea turtles, with an herbivorous diet, tend to accumulate lower concentrations of POPs compared with other wildlife.

Storelli et al. [146] measured PCBs including coplanar PCBs in loggerhead sea turtles (*C. caretta*) from the eastern Mediterranean Sea. Concentrations of PCBs in loggerhead turtles ranged from 4.65 to 52.3 ng/g ww, and the estimated toxic equivalents of non- and mono-ortho PCBs ranged from 1.54 to 5.86 pg TEQ/g ww. Alava et al. [148] measured PCBs in the loggerhead sea turtle egg yolk from North Carolina, eastern Florida, and western Florida, USA. The concentrations of PCBs ranged from 1.54 to 3,500 ng/g lw. PCB concentrations were higher in North Carolina egg samples than those in other regions, and an increasing gradient along the southeast coast around the Florida peninsula to North Carolina was found, likely due to the foraging site selection of the nesting females.

Stewart et al. [152] measured PCBs in fat and blubber of leatherback turtles (*Dermochelys coriacea*), and established baselines in blood and eggs in nesting turtles. Concentrations of PCBs in fat, blubber, blood, and egg were 4.87–188, 1.52–106, 0.162–6.54, and 0.441–19.1 ng/g ww, respectively. PCBs were found to be significantly and positively correlated between blood and eggs, suggesting maternal transfer. Camacho et al. [153] determined PCBs in green sea turtles (*Chelonia mydas*) and hawksbills (*Eretmochelys imbricata*) from the Boa Vista island, Cape Verde, Portugal. Higher concentrations of PCBs were detected in green turtles (∑PCBs 0.73 ng/g ww) than in hawksbills (0.19 ng/g ww).

Hermanussen et al. [154] investigated levels of PCDD/Fs in green sea turtles from Moreton Bay, Queensland, Australia and found higher concentrations and TEQs in turtles foraging in close proximity to river inputs. The highest levels (PCDD/Fs 580 pg/g lw and TEQs 2.7–160 pg/g lw) were elevated compared to levels reported in other higher trophic level wildlife, including seals from Greenland, Mediterranean dolphins, and Baikal seals. The results indicate that certain populations of green sea turtles that forage in close proximity to land-based secondary sources may be among the higher risk groups in terms of sensitivity to and metabolism of PCDD/Fs. Given the endangered status of many populations, future studies are needed to investigate sensitivity to and metabolism of PCDD/Fs in sea turtles.

3.4 Seabirds and Bird Eggs

3.4.1 Polar Region

Dioxins and DLCs were examined in seabirds from the Canadian Arctic (Prince Leopold Island, Lancaster Sound) [155]. Black-legged kittiwakes (*Rissa tridactyla*) collected in 1993 contained 119 pg/g lw of ∑PCDDs, 651 pg/g lw of ∑PCDFs, and 7,436 pg/g lw of non-ortho PCBs (∑NO-PCBs) in the liver. Comparable concentrations were observed in thick-billed murre (*Uria lomvia*), i.e., 169 ng/g pg lw of ∑PCDDs, 431 pg/g lw of ∑PCDFs, and 5,066 pg/g lw of ∑NO-PCBs, and northern fulmar (*Fulmarus glacialis*) contained elevated concentrations, i.e., 2,456, 7,218, and 24,067 pg/g lw of ∑PCDDs, ∑PCDFs, and ∑NO-PCBs, respectively. The ∑TEQ values were 1,117, 719, and 8,192 pg/g lw in kittiwake, murre, and fulmar, respectively. The predominant PCDD/F congener in all species was 2,3,4,7,8-PeCDF. Of the measured non-ortho PCBs, PCB-126 occurred in the highest concentrations and contributed the majority of non-ortho PCB-TEQ in all three species. Braune and Simon [155] also investigated concentration changes from 1975 to 1993, and they found that the concentrations of most PCDD/Fs decreased in the fulmars and kittiwakes but increased in the murres. Various metabolic capacities for PCDD/Fs and different migratory habits may result in those trends among species. Braune et al. [156] examined the liver tissues of northern fulmars collected in 2003 from Prince Leopold Island. The mean ∑PCDD, ∑PCDF, and ∑NO-PCB concentrations were 47.3, 154, and 519 pg/g lw, respectively, which were generally 1–2 orders of magnitude lower than the concentrations from the same species that were collected in 1993 [155]. Consequently, the ∑TEQs declined from 1975 to 2003 in northern fulmars.

In another temporal study in the Canadian Arctic (Seymour Island), ivory gull (*Pagophila eburnea*) eggs exhibited concentration declines from 1976 to 2004 [157]. ∑PCDD concentrations decreased from 207 to 55 pg/g lw, and ∑PCDF and ∑NO-PCB concentrations decreased from 61 to 24 pg/g lw and from 6,970 to 2,220 pg/g lw, respectively. The ∑TEQ also decreased from 697 to 193 pg/g lw. The ∑TEQ concentrations were greater than the no observed adverse effect level (NOAEL) (10 pg/g ww) on reproduction reported in herring gulls [158]. In the far northwestern region of Russia, peregrine falcon eggs collected from 1987 to 2001 on the Kola Peninsula contained ∑TEQ levels of 86–640 pg/g ww [159]. In Sweden, the ∑TEQ concentrations ranged from 180 to 230 pg/g lw (7.5–8.4 pg/g ww) for PCDD/Fs and from 960 to 2,000 pg/g lw (50–100 pg/g ww) for co-planar PCBs [160].

Penguin blood was tested for dioxins and DLCs in Antarctica [161]. The mean ∑PCDD and ∑PCDF concentrations ranged from 6.5 to 22 and from 2.5 to 50 pg/g ww, respectively. PCDD/Fs were generally higher in males than in females for Gentoo (*Pygoscelis papua*) and Chinstrap penguins (*Pygoscelis antarctica*), which was likely related to the partial detoxification that occurred in females during egg formation [161]. The mean ∑NO-PCB concentrations ranged from 200 to 720 pg/g

ww in three species, including Adélie (*Pygoscelis adeliae*), Chinstrap, and Gentoo penguins. PCB-126 occurred at the highest concentrations among the four measured non-ortho PCBs. The total TEQs were 21, 12, and 62 pg/g ww in Adélie, Chinstrap, and Gentoo penguins, respectively.

3.4.2 North America

Custer et al. [162] investigated PCDD/Fs and PCBs in eggs from the piscivorous belted kingfisher (*Ceryle alcyon*) from the upper Hudson River, New York, USA, and compared them with concentrations in omnivorous spotted sandpipers (*Actitis macularia*) and insectivorous tree swallows (*Tachycineta bicolor*). Total PCB concentrations in swallow eggs (with a geometric mean of 6.8 μg/g ww) were approximately half of those present in kingfishers (11.7 μg/g ww) or sandpipers (12.6 μg/g ww). However, the $\sum TEQ_{PCB}$ values were higher in swallows (1,790 pg/g ww) than in the other two species (776 and 881 pg/g ww). Sum PCDD/F concentrations and $\sum TEQ_{PCDD/F}$ values were also higher in the swallows than the other species. The authors suggested that metabolic pathway differences in the respective food chains of these three species likely accounted for the differences in the observed TEQ concentrations.

A site-specific exposure assessment of belted kingfisher was conducted in the Tittabawassee River floodplain, Midland, Michigan (USA), where the soil and sediments exhibited some of the highest levels of dioxin contamination ever reported [163]. PCDD/F concentrations were greater in belted kingfisher eggs and nestlings nesting along the Tittabawassee River when compared with those from upstream sites. The geometric mean ∑PCDD/F concentrations were 130 and 200 pg/g ww in eggs and nestlings, respectively.

Tissues from eight bald eagles (*Haliaeetus leucocephalus*) found dead in the Upper Peninsula of Michigan, USA, in 2000 were examined for contaminants [164]. Their liver PCDD/F concentrations ranged from 23 to 4,500 pg/g ww. The total TEQs ranged from 100 to 9,100 pg/g ww, of which NO-PCBs accounted for 68–88%. Some of the TEQ values were greater than the LD_{50} threshold levels reported in white leghorn chickens (115 pg/g ww) or in double-crested cormorant embryos (550 pg/g ww) [165, 166]. Eagles with elevated TCDD or total PCB concentrations tended to have high TCDD/TCDF or PCB-126/PCB-77 ratios, which may suggest an induction of cytochrome P450 enzymes and the subsequent metabolism of TCDF and PCB-77. The TEQ concentrations generally exceeded the toxicity thresholds suggested for other avian species. Bald eagles from British Columbia, Canada (1989–1994), exhibited a mean ∑TEQ of 600 pg/g ww in the liver tissues [167]. Birds with higher 2,3,7,8-TCDD concentrations tended to have low concentrations of 2,3,7,8-TCDF, indicating a hepatic cytochrome P4501A-type induction by TCDD and the subsequent metabolism of TCDF.

Great blue heron (*Ardea herodias*) eggs were collected from 1983 to 1998 along the coast of British Columbia, Canada and were evaluated for temporal changes in PCDD/Fs and PCBs [168]. The ∑TEQ-TCDD/F concentrations declined markedly

in the early 1990s, e.g., from 136 pg/g ww (1983) to 19 pg/g ww (1998) in colonies located at the University of British Columbia. The authors attributed these declines to pulp mill changes from molecular chlorine bleaching to alternative bleaching technologies and the restricted use of chlorophenolic wood preservatives and anti-sap stains. The strong positive correlation between prey fish and heron egg contaminant levels suggested that local dietary uptake was an important route of exposure for herons.

The biomagnification factors (BMFs) of PCDDs, PCDFs, and non-ortho PCBs were investigated in the fish to osprey (*Pandion haliaetus*) egg food chain in the Willamette River, Oregon [169]. The BMFs ranged from no biomagnification to 174 (OCDD). The ∑TEQ concentrations in the eggs ranged from 6 to 78 pg/g ww for PCBs, from 2 to 24 pg/g ww for PCDDs, and from 10 to 99 pg/g ww for PCDFs. The eggs of ospreys (1999–2005) nesting along the lower portion of the Columbia River, USA, exhibited a geometric mean ∑TEQ (including PCDDs, PCDFs, and PCBs) of 43.8 pg/g ww, which was significantly lower than the concentrations (mean 62.5 pg/g ww) observed in eggs collected in 1997–1998 from the same region [170]. Similar trends have also been found for many other organochlorine pesticides in osprey eggs from the studied watershed. Double-crested cormorant (*Phalacrocorax auritus*) eggs collected from the Great Lakes of North America in 1989 exhibited similar ∑PCDD/F levels across various colonies, i.e., 1,720–2,740 pg/g lw [171]. Eggs collected in 1991 from Lake Ontario contained elevated concentrations, i.e., 4,190 pg/g lw.

Dioxin-like toxic potency was also evaluated in Forster's tern (*Sterna forsteri*) eggs from Green Bay, Lake Michigan, in North America [172]. The average ∑TEQs were 214.5 and 23.4 pg/g ww from Green Bay and Lake Poygan, respectively. These data suggest that dioxin-like effects were responsible for the intrinsic reproductive problems noted in Forster's terns from Green Bay, Lake Michigan. Notably, the reported NOAEL value for reproduction in Forster's tern was 200 pg/g ww [173].

3.4.3 Europe

Jiménez et al. [174] investigated dioxins in osprey eggs collected in Spain from 1994 to 2000. The ∑PCDD/F and ∑NO-PCB concentrations ranged from 2.6 to 14 pg/g ww and from 170 to 1,390 pg/g ww, respectively. The total TEQs ranged from 16 to 140 pg/g ww. Fifty-seven percent of examined eggs contained total PCB concentrations greater than the 4 μg/g ww, NOAEL for reduced hatchability, embryo mortality, and deformities in bald eagles [175]. Eggs from yellow-legged gulls (*Larus michahellis*) and Audouin's gulls (*Larus audouinii*) collected in 2010 from the Ebro Delta Natural Park (Spain) contained ∑PCDD/Fs of 160 and 84 pg/g lw and ∑NO-PCBs of 3,100 and 6,460 pg/g lw, respectively. The mean ∑TEQ concentrations were 290 and 540 pg/g lw in these two gull species, respectively [176].

PCDD/Fs and dl-PCBs in herring gull eggs from the North Sea and Baltic Sea were analyzed [177]. The PCDD/F concentrations were 99–366 pg/g lw, and the dl-PCB concentrations were 726–2,085 ng/g lw. A general decreasing trend of PCDD/F and dl-PCB concentrations in eggs was seen between 1988 and 2003 in both locations but the relative contaminant abundance was different. In eggs from the Baltic Sea island Heuwiese, the PCDD/F concentrations were somewhat lower than those from the North Sea islands, but dl-PCBs showed higher levels in the Baltic Sea island [177]. PCB, DDTs, and several other compounds were analyzed annually in guillemot eggs from the Baltic as part of the Swedish Environmental Monitoring Program [178]. The PCB concentrations varied among species and sites; however, they had decreased temporally by approximately 5–10% per year since the end of the 1970s [178]. For example, the mean ∑PCB concentration in guillemot eggs was approximately 300 μg/g lw in 1969 and 8.7 μg/g lw in 2011 ($n = 430$). Dioxins have been retrospectively analyzed in guillemot eggs, and significant decreasing trends were observed for TCDD, TCDF, and PCDD/Fs (1970–2011), with a decreasing rate from 1.2 to 5.1% annually. However, no trend was observed between 1990 and 2011 for TCDFs. The TEQs were calculated by using the WHO_{98} TEF [1], and the geometric mean value was estimated to be approximately 2,500 pg TEQ/g lw in 1969 and 800 pg TEQ/g lw in 2011 [178].

White-tailed sea eagle (*Haliaeetus albicilla*) eggs collected along the Swedish coast of the Baltic Sea from 1992 to 2004 contained ∑PCDD, ∑PCDF, and ∑NO-PCB concentrations of 0.4–4.1, 1.2–5.3, and 180–970 ng/g lw, respectively [35], compared to ranges of 0.11–0.16, 0.22–0.33, and 57–83 ng/g lw, respectively, in the Greenland population. Non-ortho PCBs were the major contributors to the total TEQs. No evidence was found to link the reproductive impairment in eagles to the DLC concentrations in their eggs. Another study on white-tailed sea eagle eggs from Sweden reported time trends for DDTs and ∑PCBs over four decades [44]. The estimated mean concentrations of ∑PCBs decreased in sea eagle eggs from 955 μg/g lw in 1965 to 275 μg/g lw in 2010. Eggs from Audouin's gull (*L. audouinii*) and yellow-legged gull (*Larus cachinnans*) that were collected from the western Mediterranean in 1992 were evaluated for DLCs [179]. The ∑NO-PCB and ∑PCDD/F concentrations were 4,100 and 140 pg/g dw in Audouin's gull, respectively, and they were 300 and 79 pg/g dw in yellow-legged gull. The ∑TEQ concentrations were 2,955 and 126 pg/g dw in these two species, respectively. The TEQ value in Audouin's gull was much greater than the NOAEL (10 pg/g ww) for reproduction in herring gulls [158], and was comparable to the LD_{50} (550 pg/g ww) in double-crested cormorant embryos [166].

3.4.4 Asia

Piscivorous birds of various species from Japan contained dl-PCBs of 61–12,000 ng/g lw and ∑PCDD/Fs of 30–16,000 pg/g lw in the liver [180]. The dl-PCB concentrations among the species were generally in the order of omnivores > piscivores > predators > granivores that were from the same

locations. The ∑TEQ concentrations in aquatic birds ranged from 520 to 28,000 pg/g lw.

Black-tailed gulls (*Larus crassirostris*) were also used as a bioindicator of dioxin contamination in their breeding grounds in Hokkaido, Japan [181]: the mean concentrations of ∑NO-PCBs and ∑PCDD/Fs were 9,150 and 76 pg/g lw in fat and 4,905 and 53 pg/g lw in eggs. The mean ∑TEQ values were 712 and 382 pg/g lw in fat and eggs, respectively. The TEQ values reported in these studies were generally greater than the NOAEL (10 pg/g ww) on reproduction in herring gull but lower than the NOAEL (200 pg/g ww) on reproduction in Forster's tern [158, 173].

PCBs including di-, mono-, and non-ortho PCBs were studied in the livers of common cormorants (*Phalacrocorax carbo*) from Lake Biwa, Japan [182]. The calculated mean ∑TEQ-PCB concentration was 36 ng/g lw and was dominated by PCB-118, followed by PCB-126. A significant increase in ethoxyresorufin-*O*-deethylase (EROD) and pentoxyresorufin-*O*-deethylase (PROD) activities was observed in the studied cormorants, suggesting that the contamination level was sufficient to alter biochemical responses.

Dioxin and DLC concentrations were determined in the eggs, nestlings, and adults of black-footed albatross (BfA; *Diomedea nigripes*) and short-tailed albatross (StA; *Diomedea albatrus*) collected from Torishima Island in Japan in 2002 [183]. The total TEQs ranged from 1,400 to 2,900 pg/g lw in the BfA eggs and from 220 to 2,900 in the StA eggs, nestlings, and adults. The concentrations of PCDDs, with the exception of 1,2,3,7,8-PeCDD and high-chlorinated PCDFs, in 3-month-old BfA nestlings were lower than the concentrations in 1-month-old nestlings, suggesting a developmental dilution for these compounds. The estimated biomagnification factors of the examined compounds were greater in adults than in nestlings, except for 2,3,7,8-TCDF, PCB-77, Hx-CDD/Fs, and Oc-CDD/Fs. The authors hypothesized that this trend might be explained by the preferential metabolism of 2,3,7,8-TCDF and PCB-77 and the lower uptake efficiency of high-chlorinated congeners through the gastrointestinal tract in adults.

The tissue distribution of PCDD/Fs was investigated in piscivorous birds from a heavily contaminated lake (Ya-Er Lake) in China in 1997 [184]. The concentration order of PCDD/F within piscivorous birds was liver > egg ~ heart > muscle ~ stomach > brain. The highest ∑PCDD/F and ∑TEQ concentrations were 1,690 and 552 pg/g lw, respectively, in the liver. PCDD/Fs were also examined in the eggs from eight avian species in Dongting Lake, China [185]. ∑PCDD/F concentrations ranged from 21 to 4,120 pg/g lw, and the ∑TEQ of PCDD/Fs ranged from 2.5 to 17.4 pg/g lw in these species. Although the PCDD/F patterns in the eggs may be influenced by feeding habits, elimination, and metabolism, PCDD concentrations were generally greater than PCDFs in the studied species.

DLCs were examined in the subcutaneous fat of waterbirds from the Nakdong River Estuary (NRE) in Korea [186]. The mean ∑PCDD/F concentration was 396 pg/g lw in black-tailed gull (*L. crassirostris*), a resident bird of the estuary. Resident birds generally contained higher concentrations than migratory species, e.g., 198 pg/g lw in greenshank (*Tringa nebularia*), 90 pg/g lw in common gull

(*Larus canus*) and 47 pg/g lw in common tern (*Sterna hirundo*). These data suggested that the intake of locally contaminated fish near the NRE contributed substantially to the overall burdens of piscivorous birds residing in the estuary. The ∑TEQ values ranged from 34 to 227 pg/g lw in avian species.

3.5 Marine Mammals

As top marine predators, marine mammals accumulate high body burdens of POPs via feeding over a long life span and transfer large amounts to their offspring via placental and lactational transfer. Because they accumulate complex mixtures of POPs and are sensitive to their effects, marine mammals present a "real world" exposure scenario and an early warning signal about chemicals which present the greatest risk to consumers at the top of the food chain, including humans. Marine mammals include cetaceans (whales, porpoises, and dolphins), pinnipeds (phocid seals and otarids), sea otters, sirenians (manatees and dugongs), and polar bears [187]. Apart from the otters, all of these taxa have a thick layer of blubber and spend most of their time in the ocean.

Marine mammals in many parts of the world carry a plethora of POPs in their tissues including PCBs and dioxins, OC pesticides, and compounds of emerging concern such as BFRs, PFASs, PCNs, and others. While emerging contaminants have been increasing in marine mammals [188–190], PCBs remain the predominant POP in lipid tissues and pose the greatest health risks to many populations [24–26, 68, 191]. Over the past four decades, elevated concentrations of endocrine-disrupting POPs have been linked with a number of deleterious effects in marine mammals including hormonal abnormalities, skeletal deformities, reproductive failure, neoplasms, and tumors [44, 46–50, 65, 67, 68, 192, 193]. PCBs and PCDD/Fs have long been suspected to play a role in the recurring epizootics and large-scale mortalities among marine mammals, including the US Atlantic coast harbor seals (*P. vitulina*) [54, 55] and bottlenose dolphins (*T. truncatus*) [56]; seals in the Baltic and North Seas [57, 58]; Mediterranean striped dolphins (*S. coeruleoalba*) [59–61]; Baikal seals (*P. sibirica*) [62]; and Caspian seals (*P. caspica*) [63], among others. Feeding studies conducted in the mid-1980s and 1990s demonstrated the sensitivity of harbor seals to PCB and PCDD/F exposure and helped establish a threshold level for PCB-related immune suppression and other adverse effects in adult marine mammals [43, 48, 64–66]. Recent studies using a biomarker approach indicate that the threshold for PCB-induced immune- and endocrine disrupting effects in nursing seal pups is at least an order of magnitude lower [25, 67–70].

In some cases, restrictions on the production and use of PCBs and other POPs have resulted in decreasing concentrations in marine mammals, but in many parts of the world, these declines have leveled off since the mid- to late 1980s and relatively high levels of these pollutants, especially the PCBs, persist in tissues.

3.5.1 Polar Regions

A review of temporal trends for legacy POPs (PCBs, OC pesticides) in the Arctic included 316 time series in biota from marine, freshwater, and terrestrial ecosystems in Canada, Alaska, Greenland, Iceland, and Norway (including ringed seals and polar bears) [194]. Most time series show decreasing concentrations of POPs, with only a few time-series showing significantly increasing trends.

Riget et al. [195] evaluated the levels and temporal trends of PCDD/Fs and dl-PCBs in ringed seal blubber collected in 1986, 1994, 1999, and 2003 from central East Greenland. The annual median concentrations of PCDDs and PCDFs were 5.4–24.4 pg TEQ/g ww and 2.5–5.1 pg TEQ/g ww, respectively. A decreasing trend was observed for PCDD/Fs and dl-PCBs since 1986, and annual decreases were 5.2% and 5.3% for pg TEQ/g ww of PCDD/Fs and dl-PCB, respectively. In comparison, the levels of PCDD/Fs in 1986 were the highest recorded [195].

Hoguet et al. [196] assessed POP trends in beluga whales (*Delphinapterus leucas*) to determine whether restrictions on legacy POPs have led to concentration declines. PCBs were predominant contaminants in two subpopulations (Cook Inlet, Alaska, and the eastern Chukchi Sea), with median Σ_{80}PCBs concentrations of 2,360 ng/g lw in blubber. $\sum_{32}$PCBs did not change over time; however, tetra-, penta-, and hepta-PCBs decreased by 7.1, 6.8, and 8.5%, respectively, in males, whereas tetra-, penta-, and octa-PCBs declined by 11, 12, and 12.9%, respectively, in females.

Trends in POP concentrations were assessed in adipose tissues of polar bears (*Ursus maritimus*) from East Greenland between 1983 and 2010 [197]. ∑PCBs and PCB congeners (CB-153, -180, and -170/190) showed statistically significant average yearly declines of 4.4% among subadults. Mean ∑PCB concentrations declined from 22,730 ng/g lw in 1983–1986 to 8,473 ng/g lw in 2006–2010, about 2.7-fold. However, the authors concluded that despite declines resulting from international regulations, relatively high levels of these pollutants persist in East Greenland polar bear tissues [197].

McKinney et al. [198, 199] analyzed time trends of POP concentrations in adipose tissues sampled from the western Hudson Bay (WHB) polar bears. Over the 17-year period from 1991 to 2007, concentrations of ΣPCBs and Σchlordane (CHL), the two POPs at the highest concentrations in all years (>1 ppm), showed no distinct trends even when compared to previous data for this subpopulation dating back to 1968; additionally, the PCB metabolites, $\Sigma MeSO_2$-PCBs did not significantly change.

POP concentrations vary within and among circumpolar polar bear subpopulations. McKinney et al. [200, 201] measured geographic variation in PCBs in the adipose tissues of polar bears collected in 2005–2008 from 11 subpopulations in Alaska (AL), Canada including subarctic western and southern Hudson Bay (WHB, SHB), East Greenland (EG), and Svalbard (SV). ∑PCB levels were elevated relative to all other monitored POPs and increased from west to east (subpopulations means ranging from 1,797 to 10,537 ng/g lw).

Recent studies have examined the potential influence of global climate change-linked ecological changes on POP concentrations in polar bears and other arctic marine mammals (reviewed in [202]). McKinney et al. [198] first reported on climate-related changes in diet patterns in WHB polar bears, which altered time trends of POPs. Depleted carbon stable isotope ratios ($\delta_{13}C$) and shifts in fatty acid profiles, as dietary tracers, in years when the sea ice broke up earlier in the summer were proposed to be associated with higher dietary proportions of subarctic seal species (harbor seals *P. vitulina* and harp seals *Pagophilus groenlandicus*) and lower proportions of arctic seals (bearded seals *Erignathus barbatus*). When time trends were compared to those adjusted for the influence of dietary tracers, the diet change resulted in slower rates of decrease of PCBs and faster rates of increase of newer POPs. A long-term study of East Greenland polar bears showed a substantial diet shift over the past three decades, specifically, decreases in Arctic-type ringed seals and increases in subarctic-type harp and hooded seals (*C. cristata*) using a statistical approach known as quantitative fatty acid signature analysis [203]. Higher consumption of subarctic seals occurred in years of warmer temperatures and lower ice extent, as shown by associations between prey consumption and the annual North Atlantic Oscillation (NAO) Index. Climate changes may thus influence the abundance, distribution, and/or accessibility of seal prey, changing polar bear diets. Declines in PCBs were generally faster in East Greenland than in western Hudson Bay polar bears. In addition, adjusting for the proportion of ringed seal in the East Greenland polar bear diet indicated that these POP declines were not as strongly influenced by the diet as observed in western Hudson Bay.

In the Antarctic, Schiavone et al. [204] assessed DLCs in blubber, liver, and muscle of Antarctic fur seal pups. In all seal tissues, PCDF concentrations were greater than PCDDs with total PCDD/F TEQ concentrations of 150, 164, and 89 pg/g ww in blubber, liver, and muscle, respectively. These concentrations were higher than those found in previous studies of pinnipeds from the Antarctic [205, 206] possibly due to local sources on Livingston Island which hosts three permanent scientific bases.

3.5.2 North America

Trends in PCBs and dioxins have been examined in marine mammals along the US Atlantic coast since the 1970s. Lake et al. [207] reported a decrease in mean ∑PCB concentrations in blubber of harbor seals (*P. vitulina concolor*) from 17,100 ng/g lw in 1980 to 9,500 ng/g lw in 1990–1992, suggesting that PCB levels had declined significantly since the early 1970s when mean blubber concentrations in seals were approaching ~100,000 ng/g lw [208]. Shaw and co-workers [55] reported higher mean PCB concentrations in harbor seal blubber (55,000 ng/g lw in adult males; 43,000 ng/g lw in pups) [55]. Although lower than the 1970s levels, no declines in PCB concentrations in seal tissues were observed during the period 1991–2001, suggesting that the declines in PCB levels had leveled off since in the late 1980s. A similar trend was reported for European seals [209], and may reflect fresh inputs

and/or an equilibrium in environmental cycling. A recent study reported current concentrations of PCBs in liver and blubber in this population [25]. Whereas blubber is the tissue commonly analyzed, liver may be more representative of recent exposure [210]. Hepatic Σ_{30} PCB concentrations in the seals (overall mean 77,000 ng/g lw) exceeded blubber concentrations (overall mean 48,000 ng/g lw). Extremely high liver concentrations (mean 131,000 ng/g lw) were found in male pups, whereas PCB blubber concentrations were higher in the female pups (47,000 ng/g lw) than the males, suggesting possible gender differences in PCB metabolism and accumulation in young seals. Regional trends were suggestive of fresh PCB sources in industrialized, densely populated southern coast of New England versus the rural north. The data suggest that PCB concentrations in northwest Atlantic harbor seals are constant or may even been increasing in industrialized southern parts of the range, most likely due to ongoing inputs from land-based reservoirs and existing (permitted) sources [25].

Kucklick et al. [211] investigated PCBs and OC pesticides in 300 blubber biopsies from coastal and near shore/estuarine male bottlenose dolphins (*T. truncatus*) sampled along the US Atlantic and Gulf of Mexico coasts and Bermuda, and found significant regional differences in concentrations. Mean ∑PCB concentrations in dolphin blubber ranged from 33,000 to 450,000 ng/g lw among the sampling sites with the highest concentrations found in Brunswick, GA, a site heavily contaminated with the commercial PCB mixture Aroclor 1268. PCB-153, a recalcitrant congener associated with non-Aroclor 1268 formulations, was significantly associated with regional human population density, indicating this contaminant came from a general urban PCB source. Johnson-Restrepo et al. [27] examined PCB contamination in a Florida coastal food web. ΣPCB concentrations (lw basis) in biota were in the order: forage fish (silver perch, striped mullet) and Atlantic sting-rays < predator fish (red drum, hardhead catfish, spotted sea trout, spiny dogfish) < Atlantic sharpnose sharks < bull sharks and bottlenose dolphins. PCB concentrations in sharks and dolphins were one to two orders of magnitude greater than those in the lower trophic level fishes. The biomagnification factors (BMFs) for ΣPCBs, calculated as the ratio between lipid-normalized concentrations in predator and prey, ranged, on average, from 3 to 502, indicating a high potential for biomagnification in this food web. The highest BMFs of ΣPCBs were measured from forage fish (silver perch) to bottlenose dolphins and bull sharks (*Carcharhinus leucas*). Bull sharks are apex predators that inhabit estuarine, near-shore, and offshore waters of both the Gulf and the Atlantic coasts of Florida. These sharks are the only shark species to penetrate far into freshwater habitats.

On the US Pacific coast, She et al. [212] measured PCBs and DLCs in harbor seals from the San Francisco, CA area between 1989 and 1998. Overall, PCDD/F concentrations were low in these seals, but concentrations of non-ortho PCBs were relatively high (693 pg/g lw; 68 pg TEQ/g lw). The mean ΣPCB concentration in seal blubber was 71,000 ng/g lw, which is comparable to levels reported for Atlantic coast harbor seals. Pacific killer whales (*Orcinus orca*) living in the waters along the northwest coast of the USA and Canada are among the most contaminated marine mammals in the world. Ross et al. [45] measured PCDD/Fs and PCBs in

blubber biopsies from the northern resident, southern resident, and transient populations of killer whales from the region, and found significant differences in PCB contamination among the three populations. The transient whales had the highest levels (mean adult male: 251,000 ng/g lw) followed by the southern residents (mean AM: 146,000 ng/g lw), with lower levels in the northern residents (mean adult male: 37,000 ng/g lw). Within each population, higher levels were found in the adult males compared with the females. The authors suggest that the contamination difference among the populations may partly be a result of trophic level and dietary differences. Resident populations feed mainly on fish such as salmon, while offshore transient killer whales feed mainly on marine mammals, e.g., sea lions [45].

3.5.3 South America

Dorneles et al. [26] analyzed PCBs and dioxins in blubber of false killer whales (*Pseudorca crassidens*), Guiana dolphins (*Sotalia guianensis*), rough-toothed dolphins (*Steno bredanensis*), and in liver of franciscana dolphins (*Pontoporia blainvillei*) collected from southeast and southern Brazil. Dl-PCBs accounted for over 83% of the total TEQ for all cetaceans. Total DLC concentrations ranged from 36 to 3,006 ng/g lw for franciscana dolphins, and from 356 to 30,776 ng/g for other delphinids. Mean ΣPCB concentrations ranged from 35,000 to 279,000 ng/g lw, indicating that these cetaceans are highly contaminated, on par with the high PCB levels in transient Pacific coast killer whales. The high concentrations found in the study raised concern not only about the conservation of Brazilian coastal cetaceans, but also regarding possible human health risks from consumption of fish from Brazilian estuaries.

3.5.4 Europe

In 1990, the UK started its Cetacean Strandings Investigation Programme to gain greater understanding of contaminant levels in its marine mammal population. In an update on contamination status between 1990 and 2008, Law et al. [191] report sum PCB concentrations in harbor porpoise blubber ranged from 48 ng/g lw to 160,000 ng/g lw. Long term trends show an early decline in PCB concentration that has plateaued since about 1998. Different regions of the UK show somewhat different patterns. PCB concentrations have been steady in the East, variable around Scotland, and steadily decreasing in the West.

Storelli et al. [213] measured PCBs including dl-PCBs in melon, blubber, liver, kidney, lung, heart, and muscle tissue of striped dolphins (*S. coeruleoalba*) from the Eastern Mediterranean Sea (Adriatic Sea). The PCB concentrations ranged from 7 to 69,822 ng/g ww in the organs. Blubber and melon had the highest concentrations (22,000 and 16,400 ng/g ww, respectively) followed by liver (3,600 ng/g ww), and the other organs (mean range 220–725 ng/g ww). Total Dl-PCB TEQ in blubber

was 120,000 pg/g ww, 19,900 pg/g ww, and 21,000 pg/g ww in adult males, adult females, and newborns, respectively. Both the blubber PCB concentrations and the Dl-PCB TEQs exceeded estimated toxic thresholds for adverse effects in harbor seals [43, 45] thereby indicating that this population is likely under stress.

Imaeda et al. [214] investigated PCDD/Fs, PCBs, and dl-PCBs in the blubber of Baikal seals collected in 1992 and 2005. Dl-PCBs were one of the dominant contaminants, with concentrations ranging from 480 to 3,600 ng/g ww. Concentrations of PCDDs and PCBs in males were significantly higher than in females. In males, age-dependent accumulation was observed for PCDDs and mono-ortho PCBs, but PCDFs and non-ortho-PCBs showed no such trends implying that the seals may preferentially metabolize these contaminants or that exposure has been decreasing in recent years. Concentrations of PCDFs and non-ortho PCBs were significantly lower in 2005 than 1992, but no decreasing temporal trend of PCDDs, mono-ortho PCBs, or most non-dioxin like PCBs was observed. In 2005, TEQ levels in 40% of the specimens exceeded the threshold level for adverse effects in harbor seals (209 pg/g ww) [214], which raises concern for the future of the population.

3.5.5 Asia

Moon et al. [215] measured PCDD/Fs and dl-PCBs in the blubber of finless porpoises (*Neophocaena phocaenoides*) collected from Korean coastal waters. Total TEQ concentrations for PCDD/Fs and dl-PCBs were 6.5–31 pg/g lw, which were lower than those of cetaceans and pinnipeds reported from other countries and below the suggested threshold values for adverse health effects in marine mammals. Moon et al. [216] also measured PCDD/Fs and dl-PCBs in liver and blubber of minke whales and long-beaked common dolphins. It showed that PCDF and dl-PCB concentrations in blubber were 3–10 times higher than those in liver, but PCDDs were higher in liver. Concentrations of PCDD/Fs and dl-PCBs in liver and blubber of dolphins were significantly higher than those in whales, due to differences in habitat and diet [216]

Yang et al. [217] determined PCDD/Fs and PCBs in the blubber, liver, kidney, stomach, small intestine, and brains of Yangtze finless porpoises (the sole freshwater subspecies of finless porpoise) from Dongting Lake, China collected from 1998 to 2004. The results showed PCDD/F concentrations ranged from 65 to 1,563 pg/g lw in the organs, and PCBs ranged from 60 to 1,890 ng/g lw.

In 1998, Noël et al. [218] collected blubber biopsy samples from killer whales (*O. orca*) inhabiting the coastal waters around Possession Island, Crozet Archipelago, southern Indian Ocean, for contaminant analyses. The results showed that PCDD concentrations ranged from 5 to 77.1 pg/g lw, PCDFs ranged from 7 to 36.1 pg/g lw, and PCBs ranged from 4.4 to 20,500 ng/g lw. Over 70% of killer whales had blubber PCB concentrations above the PCB threshold established for endocrine disruption and immunotoxicity in young harbor seals, suggesting that PCBs cannot be ruled out as a threat to this declining population [218].

3.5.6 Maternal Transfer

POP concentrations in adult female marine mammals are generally lower than those in adult males due to the transfer of contaminants from females to their offspring during gestation and lactation, except in highly contaminated areas where females maintain higher body burdens due to ongoing exposure [45, 59, 219, 220]. Concentrations of a large suite of POPs were recently examined in the blubber and serum of juvenile and adult Hawaiian monk seals (*Neomonachus schauinslandi*) from the main Hawaiian Island subpopulation [221]. Adult females have the lowest blubber levels of most POPs, whereas adult males have the highest levels [221]. In contrast, a recent study showed that the blubber PCB concentrations in Tasmanian long-finned pilot whales (*Globicephala melas*) decreased with age in males because of growth dilution effect or decreasing levels of PCBs in the environment [222]. POPs were investigated in matched liver samples from five mother–fetus pairs of gray seals (*Halichoerus grypus*) [223], in blubber samples from 20 female sea lions and their fetuses during late pregnancy [224], and in Alaskan harbor seals (*P. vitulina*) [225]. Significant amounts of PCBs and other POPs were transferred from female harbor seals to their fetuses during pregnancy and distributed among the fetal organs [225]. The prenatal transfer of these toxic contaminants may pose health risks to the fetus during early development.

Lactational transfer of PCBs and other POPs was examined in gray seal mother–pup pairs from Scotland [226, 227]. Generally, concentrations of all contaminants increased in the mother and pup tissues from early lactation to late lactation. Mobilization of contaminants from the maternal inner blubber layer to the bloodstream was more efficient for less lipophilic compounds (lower $\log K_{ow}$) than for more lipophilic compounds, leading to selective transfer of lower $\log K_{ow}$ congeners to the pups. Exposure of young marine mammals to toxic contaminants both in utero and during nursing can lead to very high burdens in their developing bodies. Because of their greater sensitivity to developmental toxicity, Mos et al. [68] proposed a much lower toxicity reference value for PCB contamination (1.3 μg/g lw) for pups than had been previously estimated for immunotoxicity in adults (17 μg/g lw [43]).

4 Health Effects and Risk

4.1 Endocrine Disruption

Dioxins and PCBs affect the health of wildlife and their progeny by interfering with their endocrine system, which is responsible for maintaining homeostasis, reproduction, development, and/or behavior [40, 228]. Thus, dioxins and PCBs are known as endocrine disruptors (EDs). The magnitude of adverse effects depends

on the body burden, dosage, frequency, and duration of exposure at different life stages [229]

Thyroid hormones play an important role in growth and development (including somatic and brain development) and in the maintenance of normal physiological status in vertebrates. Dioxins and PCBs can interfere directly with hormone synthesis in the thyroid gland [230–232], and disrupt thyroid hormone receptors and accessory proteins that directly control gene expression through the thyroid hormone responsive element [232]. Additionally, these pollutants competitively bind to thyroid hormone transport proteins in blood, such as transthyretin (TTR), and to membrane-bound transporters of target cells or to intracellular cytosolic thyroid hormone binding proteins, which are thought to act as modulators of nuclear-receptor-mediated transcription [232].

Brar et al. [233] studied thyroid endocrine-related effects and their relationship to accumulated contaminants in two indigenous fish species sampled from San Francisco Bay. Total triiodothyronine (T3) and total thyroxine (T4) levels varied significantly by location, with differing T3/T4 ratios in fish from some locations indicating altered peripheral deiodinase activity. The changes in levels of thyroid endocrine hormone were significantly correlated with hepatic concentrations of certain environmental contaminants. Exposure to a large number of polychlorinated biphenyl (PCB) congeners, both dioxin-like and non-dioxin-like, showed significant inverse correlations with T4 levels in the fish, while in contrast, T3 and T3/T4 ratio were positively correlated with PCB exposures. The positive correlation between T3/T4 ratio and PCB exposure supports the hypothesis that PCBs may alter T4 deiodination.

Similar results were found among northern fulmar (*F. glacialis*) populations from the Canadian Arctic and northern Europe [234]. Hepatic concentrations of dioxins, furans, and DL-PCBs were amongst the highest ever reported in northern seabirds. Hepatic EROD activity and plasma T4 levels were positively correlated with liver organochlorine levels, particularly with the dioxin-like compounds. Additionally, strong negative correlations were found between the dioxin-like compounds and plasma T3 levels. This study provides additional evidence that PCBs, dioxins, and furans may be associated with thyroid disruption [234].

Janz and Bellward [235] examined the effects of *in ovo* TCDD exposure on plasma thyroid hormone concentrations (T3, T4) and body and skeletal growth during the perinatal period in the domestic chicken (*Gallus gallus*), domestic pigeon (*Columba livia*), and great blue heron (*A. herodias*). They found that although hepatic EROD activity was induced 13- to 43-fold above controls in chicken, there was no effect of TCDD exposure on hatchability, body growth, subcutaneous edema, or plasma thyroid hormone levels. For pigeons exposed to TCDD, EROD activity was induced 6- to 15-fold, hatchability was decreased, liver to body weight ratio was elevated, and body and skeletal growth were decreased ($p < 0.01$); but there was no effect of TCDD exposure on plasma thyroid hormone levels. For herons, hepatic EROD activity was induced two- to threefold above control birds, similar to EROD activities measured in heron hatchlings exposed to environmental levels of TCDD and related chemicals in the Strait of Georgia,

British Columbia. But at this level of TCDD exposure, there was no observed effect on plasma thyroid hormone levels or body growth in herons [235].

Field evidence has suggested that plasma PCBs and thyroid hormones are correlated in polar bears, apex predators in the Arctic food web [236]. Amongst females, there were significant correlations between five thyroid hormone variables and plasma PCB levels, but among males, PCBs were related to only two thyroid hormone variables, suggesting that female polar bears may be more susceptible to PCB-related thyroid hormone alterations than are males. In female polar bears from Svalbard, Norway, higher PCB concentrations were positively correlated with increasing plasma progesterone levels, which may indicate possible a defeminizing effect via inhibition of enzymes that convert progesterone to estrogen [237]. In Greenland polar bears, higher PCB and OC levels were negatively correlated with bone mineral density, suggesting a similar anti-estrogenic effect of the compounds [238, 239].

4.2 Developmental/Reproductive Effects

Dioxins, PCBs, and many other DLCs are estrogenic and may adversely affect reproductive functions in diverse species of biota. An abundance of evidence from laboratory and field studies suggests that DLCs can cause infertility [240, 241], reduced hatch rates in fish and birds, and decreased offspring viability in addition to altered hormone levels and adult sexual behaviors [242]. One example is the estrogenic induction of vitellogenin (Vtg) in fish [243]. Vtg is a complex phospholipoglycoprotein synthesized by the liver in response to estrogen stimulation. Vtg is secreted by the liver and transported in the blood to the ovary, where it is sequestered and cleaved into the yolk proteins lipovitellin and phosvitin, which are stored in the yolk and serve as a food reserve for the developing embryo [244]. High Vtg levels in male fish are associated with liver enlargement, feminization, and kidney damage, and are generally accompanied by various degrees of reproductive interference at similar or lower ambient estrogen concentrations [245–248]

Field studies have been conducted to address ecotoxicological concerns regarding PCB exposure in snapping turtles (*C. serpentina*). Eisenreich et al. [140] examined sublethal and lethal responses of juvenile snapping turtles that were exposed maternally and/or through diet to PCBs over 14 months post-hatching. Maternal exposure did not affect embryonic development or hatching success. Dietary PCB exposure reduced the metabolic rates of juveniles in two of the three assays. Kelly et al. [141] reported the accumulation and maternal transfer of PCBs in snapping turtles from the upper Hudson River, NY, USA, by using eggs and blood samples. Significant positive correlations were found between the carapace length and blood PCB concentrations for both sexes in contaminated areas. The results suggest that maternal transfer of PCBs to snapping turtle eggs and high body burdens pose reproductive risks to turtles in the upper Hudson River area.

White-tailed sea eagles had very low population numbers in the 1980s which was linked with high levels of organochlorines (PCBs and DDT) [44, 52, 53]. However, since the banning of DDT and PCBs in the 1970s, reproductive health and population numbers have increased and DDT and PCB concentrations have decreased at fairly similar rates. The mean productivity of sea eagles increased from 0.3 in 1965 to 1.0 in the mid-1990s. Apart from eggshell thinning caused by DDE, PCBs have been correlated with impaired sea eagle reproduction, implying increased embryo mortality in eggs with elevated PCB concentrations [249]. High levels of PCB and DLCs were associated with adverse effects on reproduction and development in other avian predators including Alaskan peregrine falcons, bald eagles from the Aleutian Islands, glaucous gulls from Bjørnøya (Norway), and great black-backed gulls from northern Norway [237, 250].

Organochlorines (primarily PCBs) were suspected to be the underlying cause of reproductive failure in Baltic otters in the 1970s when reproduction plummeted to approximately 2% [52]. Following PCB and DDT bans in the Baltic region, the frequency of adult female otters with signs of reproduction slowly recovered to approximately 67% in 2010 [44, 52].

Similarly, PCBs and DDT were implicated in the widespread reproductive impairment and population declines among seals from northwestern Europe [48, 51, 251, 252] and the disease complex observed in Baltic seals [47, 253]. Because of high hunting pressure, the populations of gray and ringed seals in the Baltic decreased dramatically from 1,900 to approximately 1,950 [254]. Even after their protection, a further decrease followed and a disease complex, including uterine obstructions and tumors (leiomyomas) leading to impaired reproduction, was described [251, 253]. The disease complex was found at high frequencies among gray seals in the 1970–1980s [255], and a high contaminant concentration in their prey was proposed to be the underlying cause. PCBs were indicated as the primary suspect in the reproductive failure [47, 251] and were associated with tumors found in the uteri of older females [256]. Approximately 70% of the females had uterine obstructions in the early 1970s, but the frequency decreased after the end of the 1980s. The pregnancy rate in examined seals increased from close to zero in the late 1970s to close to 100% today. The first signs of recovery were observed approximately 15 years after the ban of PCBs and DDT [44, 52, 53].

An early feeding study conducted by Reijnders [48] reported that reproductive failure in harbor seals from the Wadden Sea was linked to consumption of PCB-contaminated fish. This study was the first to demonstrate a causal relationship between contaminant levels and a physiological response in marine mammals. Subsequent feeding studies conducted in the Netherlands showed that harbor seals that consumed contaminated fish from the Wadden Sea or the Baltic Sea exhibited increased infertility and immunosuppression compared with seals consuming less-contaminated Atlantic fish [48, 64, 65].

4.3 Immunotoxicity

Although dioxins and PCBs are well-studied immunotoxicants in animals [257, 258], little attention was paid to this aspect of their potential toxicity in wildlife until the 1980s when a series of disease outbreaks resulted in mass mortalities among marine mammals, suggesting that the animals were susceptible to disease via PCB-induced suppression of their immune systems [45, 61, 259, 260]. A weight of evidence suggests that PCBs exert immune, endocrine disrupting, and reproductive effects in marine mammals, and PCB toxicity has been strongly implicated in the epizootics that have decimated many populations since the 1980s [43, 45, 68, 261].

Harbor seals in polluted regions of Europe have experienced several virus-induced mortalities, starting with the 1988 morbiilvirus outbreak that resulted in the deaths of 20,000 animals [58, 262]. Similar to European harbor seals, the northwest Atlantic harbor seal population has been susceptible to viral disease outbreaks, as evidenced by a recurrence of epizootics since the late 1970s. In 1979–1980 and again in 1991–1992, viral epizootics resulted in the deaths of approximately 1,000 harbor seals from Maine to New York [54, 263, 264]. Between 2004 and 2009, approximately 2,000 harbor seals died of unknown causes during "Unusual Mortality Events" (UMEs) along the New England coast [265]. The possible contributory role of PCBs and DLCs in these events cannot be ruled out, since the PCB burdens alone in these seals exceed the estimated threshold levels for POP-mediated reproductive and immune system effects in the species [25, 43, 55].

PCB-induced immunotoxicity has been implicated in several dolphin mortalities, rendering the animals susceptible to bacterial, viral, and parasitic infections. In 1987–1988, more than 740 bottlenose dolphins (*T. truncates*) stranded along the northwest Atlantic coast from New Jersey to central Florida [266]. It is estimated that this population may not return to pre-1987 population levels for 100 years [266]. Other similar large-scale dolphin mortalities have been reported in the Gulf of Mexico and in the Mediterranean Sea [60, 267].

Captive feeding studies conducted in the 1990s showed that harbor seals exhibited depressed immune responses if they consumed contaminated fish from the Baltic Sea rather than cleaner fish from the Atlantic Ocean [65, 66, 268]. An impairment of natural killer cell activity, in vitro T-lymphocyte function, antigen-specific in vitro lymphocyte proliferative responses and in vivo delayed-type hypersensitivity and antibody responses to ovalbumin were observed in the seals fed contaminated Baltic fish [65]. Similar feeding studies were conducted in European otters and mink [240, 241, 269, 270]. A study of northern fur seals found that various immune function parameters were negatively correlated to increasing PCB levels, including decreased antibody production after vaccination [271]. In Svalbard polar bears, a significant decrease in antibodies was associated with increased PCB levels [272]. A similar negative correlation was found between IgG levels and increasing PCB levels in the cubs. In the Svalbard/Resolute study, polar bears with high PCB levels exhibited immunosuppression expressed as

reduced IgG production and lowered lymphocyte responses after vaccination [273]. These studies have provided valuable information for establishing a dose–response relation between PCB exposure and adverse health effects for aquatic and marine mammals [43].

Based on the data from field and captive feeding studies in marine mammals and mink, Kannan et al. [43] proposed thresholds for immune and reproductive effects of ΣPCBs in marine mammals of 8.7 μg/g lw in the liver and 17 μg/g lw in the blubber. Recent studies have applied these thresholds to estimate potential toxicity of current body burdens of POPs in marine mammals [191, 274]. In UK harbor porpoises with blubber ΣPCB concentrations above 17 μg/g lw, those that died of infectious disease had higher ΣPCB concentrations than those that died of traumatic injury, whereas there was no relationship between PCB concentrations and cause of death for porpoises with PCBs below the threshold [191].

Results of recent biomarker studies of PCB-sensitive endpoints in young free-ranging harbor seals such as decreased immune function, vitamin A, and thyroid hormones suggest that PCB-related adverse effects occur at much lower exposure concentrations in pups than previously demonstrated [67, 68, 70, 192, 275, 276]. Using No Observed Adverse Effect Levels (NOAELs) and allometrically scaled toxicity reference values (TRVs) from rodent studies, Mos et al. [68] proposed new TRVs (consisting of 5% tissue residue concentration and dose) of 1,300 ng/g lw Σ_{154}PCB in the blubber of nursing harbor seal pups. These new TRVs were applied in a recent study of harbor seals along the northwest Atlantic to determine potential PCB-related immunotoxicity and other sublethal effects in the pups [25]. Blubber Σ_{30}PCB concentrations in 87% of the pups exceeded the threshold of 1,300 ng/g lw by an order of magnitude, implying that the majority of these pups may be suffering from PCB-related adverse effects that could diminish their overall fitness and increase their susceptibility to infectious disease during a vulnerable stage of development.

Given the pattern of recurring epizootics among seals and other marine mammal species, current tissue concentrations of PCBs and DLCs may pose a significant immunotoxic threat to the future health of many populations.

4.4 Population Effects

It is difficult to associate a specific compound or class of compounds with a specific health effect in the wild because there are thousands of contaminants in circulation, and many other biotic and abiotic factors may influence animal health. Because of the difficulty in making quantitative observations in the field, it is not easy to identify the types of disturbances that cause wildlife populations to be potentially at risk or reproductively compromised. Any causative mechanisms for altered reproductive parameters or population declines in aquatic biota are likely to be multifactorial and may involve a complex interplay of factors such as contaminant stress, habitat loss, accidents, noise, and climate change [74, 277, 278]. However,

risk assessment can still be reasonably performed using a combination of approaches, including feeding studies, biomarker studies, weight of evidence, and extrapolation across species (e.g., from mink to marine mammals).

Chronic exposure to PCBs and DLCs impacts the fitness and sustainability of many wildlife populations today, especially apex predators such as sea turtles, sharks, and marine mammals [27, 45, 279, 280]. Overall, marine mammal health has been deteriorating over several decades, and trends in marine mammal disease reports are rising [174, 281]. In some regions such as the Baltic where levels of PCBs and OCs have been banned, the reproductive health of seals has gradually improved, and seal population numbers have increased [282]. A similar pattern has been observed for otters and white-tailed sea eagles [44].

The impact of dioxin and dl-PCB contamination on the reproductive health of populations of fish-eating birds including gray herons (*Ardea cinerea*), great cormorants (*P. carbo*), osprey (*Pandion halieaetus*), and kingfishers (*Alcedo atthis*) in the Tokyo Bay area, Japan, has been a concern, although environmental levels have been decreasing [283]. A regional assessment of the ecological risks of dl-PCB exposure was performed to assess the need for risk management measures to protect these populations [283]. Egg mortality risk related to the contamination levels of dl-PCBs in eggs was determined to be relatively low (from <1 to 12%), as were the changes in the population growth rates (lambda) (<1 to 2%), indicating that the current levels of dl-PCB contamination alone are not sufficient to trigger population-level effects.

However, some regions are still highly polluted and populations of high trophic level wildlife continue to suffer from disease and mass mortalities, in some cases, reducing population numbers to levels that cannot be sustained. It is estimated that more than one-third of marine mammals in the North Atlantic and Pacific are in danger of going extinct, according to the International Union for Conservation of Nature (IUCN) [280]. After accidental mortality, pollution is ranked as the prevalent cause of disease and death, affecting up to 60% of all marine mammals. Although it is arguable whether dioxins and PCBs are the primary cause of the mass mortalities affecting marine mammals and other top predators, these compounds have the potential to exacerbate the magnitude of disease outbreaks by compromising normal immune resilience in the animals [45, 284]. For many contaminant-stressed populations, the added stress of climate change is exacerbating the problem, causing shifts in food webs and threatening the survival of species, not only in the Arctic but in temperate oceans as well [280].

Vulnerable species such as polar bears living at the edge of their physiological tolerance range are at extreme risk. Of 19 subpopulations comprising some 25,000 polar bears, at least three are declining rapidly [285]. Canada's Western Hudson Bay (WHB) population has experienced a 22% decline or greater since the early 1980s; the Southern Beaufort Sea population plunged by about 40% over a 10-year period from 2001 to 2010, dropping from about 1,500 bears to 900 bears before stabilizing; and the Baffin Bay population, shared by Greenland and Canada, is in steep decline [286]. The main factor driving these declines is the melting of Arctic sea ice, averaging 10% per decade, displacing the habitats and prey of polar bears,

beluga whales, and walruses. But ironically, climate change alterations in food webs, lipid dynamics, ice and snow melt, organic carbon cycling, and severe storm events are expected to increase both the distribution and toxicity of POPs in coastal and oceanic environments [277]. Many scientists fear that this will trigger even larger-scale catastrophic events among marine mammals. The complex interplay of contaminant stress, climate change, and shifts in food web dynamics on vulnerable populations is an area urgently needing further research.

All seven species of marine turtles have been classified as threatened or endangered, and populations have declined steeply (by almost 95%) in recent years [287]. The causes are unknown, but sea turtles face multiple threats from diverse hazards such as trawler nets, egg poaching, and pollution. Turtles are known to be robust to physical damage, but are surprisingly very susceptible to chemical contaminants [147]. An additional stress on populations is the expansion of fibropapillomas (FP), a benign neoplastic disease associated with a turtle herpesvirus that has increased significantly over the past 20 years [288]. FP is a global disease affecting turtles worldwide including the green sea turtle (*C. mydas*), the loggerhead (*C. caretta*), olive ridley (*Lepidochelys olivacea*), kemp's ridley (*Lepidochelys kempii*), and leatherbacks (*D. coriacea*) [288]. The disease tends to develop after the open-water pelagic developmental phase of approximately 10 years when juvenile turtles arrive and forage on seagrass and algae in nearshore environments with relatively small home ranges. Studies have linked a high prevalence of tumors in turtles to environmentally disturbed habitats, e.g., heavily polluted coasts with high human population density, proximity to river inputs, agricultural runoff, and land-based sources [289]. Immunosuppression is also strongly correlated with FP, but may be a consequence of the development and growth of FP, similar to other virus-induced tumors in other species [288]. The possible role of immunotoxic contaminants such as PCBs and dioxins in sea turtle disease and population declines is not understood and warrants further study.

5 Conclusions

5.1 Data Gaps

From four decades of field and semi-field (feeding) studies, we have a better understanding of the sources, exposure pathways, environmental levels, trends, and health effects of dioxins, PCBs, and related DLCs in aquatic ecosystems. However, data are lacking for many regions, contaminant patterns are changing over time, and large research gaps remain.

- Data are lacking for biota in many highly populated developing countries where the problems of inadequate waste management infrastructure and high volume of waste production are growing larger. Limitations in technical and financial support in these countries may exacerbate the problem. For aquatic biota from

war-torn regions of the Middle East and Africa, contaminant data are non-existent.

- Many developing countries are still establishing contaminant inventories, which are essential before any technologies and measures can be put in place for source reduction. Bridging the data gap between developed and developing countries would provide a clear picture of the needs and options for effectively reducing global contamination.
- The rise of new and emerging persistent pollutants, poses a more complex global threat to both wildlife and humans. While it is known that dioxins and PCBs are linked to cancers, reproductive impairment, and immune system dysfunction in animals, more studies are warranted to understand the extent of the compounded health threat posed by the introduction of emerging POPs.
- Extrapolating from recent trend studies, PCBs remain the predominant contaminants in fish, seabirds, turtles, sharks, and marine mammals, but in certain food webs, bioaccumulation and magnification of PCDD/Fs and dl-PCBs are evident, presenting an increased health risk to marine animals. The synergistic interactions and effects due to exposure to contaminant mixtures in aquatic biota need further investigation.
- Technology has been advancing at an exponential rate, and it is important to use advances in analytical chemistry to advance our science. The evolution of electronic tracking tags might provide an alternative for tracking long-distance migrating species [290].
- Data quality is crucial to decision-making leading to resource management and contaminant regulation. Standardization of sampling, storage, and analytical protocols and harmonization of analytic methods could improve the integrity of collected data across the studies. Additionally, an international, public database for reporting POP levels and mass mortalities in wildlife might facilitate scientific discovery and allow for timely response to warning signals from contaminant-stressed populations.
- Biological effects of dioxins and PCBs are generally first observed in populations with high-level exposure, often accompanied by catastrophic events such as reproductive failure or disease outbreaks. More biomarker studies are needed to advance understanding of the range of health effects associated with moderate to low-level exposure, especially in species and populations in decline, e.g., sea turtles. The possible role of immunotoxic PCBs and dioxins in sea turtle tumors (FPs) and declining numbers worldwide is not understood and warrants further study.
- Climate change alterations in food webs, lipid dynamics, ice and snow melt, organic carbon cycling, and severe storm events are expected to increase both the distribution and toxicity of POPs in coastal and oceanic environments [277]. Many scientists fear that this will trigger widespread, large-scale catastrophic events among marine mammals and other high trophic level wildlife. The complex interplay of contaminant stress, climate change, and shifts in food web dynamics on vulnerable populations such as polar bears is an area urgently needing further research.

5.2 Foresight from Current Knowledge

Dioxins, PCBs, and DLCs are major industrial chemicals that are globally restricted, but still pose a significant health threat to diverse species of aquatic biota, particularly those at high trophic levels. Although PCBs were banned in most developed countries four decades ago, landstocked sources such as old PCB-containing equipment and landfills will remain a reservoir for PCB releases for years to come. Tanabe [291] predicted that most of the PCBs (66%) were stockpiled in products (transformers and capacitors) long after PCBs were banned from production in the 1970s. Trend studies suggest that the amounts of PCBs cycling in aquatic and marine food webs are gradually decreasing, as burial, metabolism, and degradation occur in sediments. However, certain uses of PCBs are still permitted in the USA and Europe, delivering fresh inputs to aquatic food webs. As a result, the large declines observed in PCBs after the bans have plateaued in most developed countries, and in some regions, environmental levels are remaining constant or even increasing. Similarly, regulatory actions aimed at controlling PCDD/Fs and PBDD/Fs have been inefficient, as these compounds continue to be released as by-products of combustion and other industrial and agricultural processes.

With the ongoing challenges associated with POP contamination, continuous efforts are needed to monitor the occurrence, distribution, and health effects in biota with emphasis on highly exposed species with body burdens close to or higher than estimated threshold levels of effect. Thresholds with high uncertainties should be interpreted as warning signals that require more data and further study.

Overall, the future trends for dioxins, PCBs, and related DLCs in aquatic biota are unclear. Given the large reservoir of these compounds in the environment, and ongoing releases and inputs from permitted sources, regulatory controls will do little to impede the continued cycling of these chemicals through aquatic and marine ecosystems for the foreseeable future.

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Exposure, Bioaccumulation, Metabolism and Monitoring of Persistent Organic Pollutants in Terrestrial Wildlife

Shane R. de Solla

Abstract Despite the reduction in the production and emissions of legacy organohalogenic compounds, such as polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides and polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), these persistent organic pollutants (POPs) are still amongst the most relevant found in wildlife tissues. With some exceptions, POPs are very lipophilic, and thus the movement of dietary lipids drives the movement, distribution and sequestering of these compounds in wildlife tissues. Other avenues of exposures, however, can also be important in special circumstances, such as inhalation and dermal exposures. Although these POPs are relatively resistant to metabolism, the rates of metabolism are generally the rate-limiting step for their elimination and contribute to substantial differences in body burdens amongst species. The capacity for metabolizing POPs is highest in mammals, on average slightly lower for birds but much lower for ectothermic vertebrates. Nonetheless, differences in metabolic ability for specific enzymes, such as cytochrome P450, in related taxonomic groups are sufficiently large to show differences in compound-specific accumulation. Due to the reduction in the production and emissions of legacy POPs, in general the body burdens in wildlife have declined worldwide. Despite that, concentrations of POPs are still elevated in many wildlife populations, particularly higher trophic level predators. The rates of elimination of POPs from body burdens of wildlife are much higher than the much slower rates of environmental degradation; hence, the changes in body burdens better reflect any changes in the bioavailability of POPs. Lastly, some of the advantages of using wildlife as monitors of POPs are discussed.

Keywords Bioaccumulation, Exposure, Metabolism, Monitoring, Persistent organic pollutants, Wildlife

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Contents

1 Introduction

Legacy contaminants are named, ostensibly, because they are no longer being produced, which implies that the exposure of wildlife to these contaminants is not only decreasing but that there is some justification for the substantial shift in priorities from studying legacy compounds to emerging compounds. Certainly, research and monitoring of newer classes of compounds, particularly those in production or being traded in commerce, is necessary to be able to assess the risks and hazards of these newer chemicals to the environment, to fish and wildlife and to human health. Nonetheless, it should be emphasized that, after 40 years following the almost-near cessation of the production of polychlorinated biphenyls (PCBs) in North America, and the rapidly declining global production elsewhere, PCBs are still generally the most abundant persistent organic pollutant (POP) found in wildlife tissues, with the importance of organochlorine pesticides not far behind. Although body burdens of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) are much lower than those of other legacy compounds, and even emerging

compounds such as brominated flame retardants and perfluorinated compounds, few compounds are as acutely or chronically toxic as are dioxins. Furthermore, aside from PAHs and metals, emerging compounds rarely exceed sediment, soil or tissue guidelines and trigger expensive remedial actions, which PCBs and related compounds often do. Hence, the relatively high concentrations highlight the importance of considering these legacy compounds in monitoring programmes of environmental levels or assessments of wildlife health.

This chapter will examine traditional persistent organic contaminants, such as polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides and polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs). By definition, persistent organic pollutants (POPs) are "...organic compounds that, to a varying degree, resist photolytic, biological and chemical degradation", [1]. These groups of compounds are unrelated in their expected end use and have been produced to be used as electrical insulators and compounds designed to kill pests and act as flame retardants or are accidental by-products of the purposeful manufacturer of structurally related halogenated organic compounds. The focus of this chapter will track from the exposure of wildlife to legacy POPs, through bioaccumulation and elimination, and finally to the monitoring of POPs in wildlife.

2 Legacy Contaminants

2.1 Polychlorinated Biphenyls (PCBs)

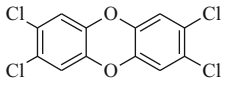
Polychlorinated biphenyls (PCBs) were used first as lubricants, electrical insulators, heat transfer fluids and surfactants. They were most commonly used in transformers, capacitors and hydraulics but were also found in a very wide range of products such as sealants, flame retardants and plasticizers. They were useful because they were stable at high temperatures and were excellent electrical insulators. Although PCBs were first synthesized in 1881 [2], they were first produced commercially by 1929 by the Swann Chemical Company, until Monsanto took over production in 1935. North American production peaked in the early 1970s, but by 1972 North American use of PCBs was limited to "closed systems" (i.e. products in which the PCBs were entirely contained). Production in North America ended in 1977, but production in Europe (e.g. France and Spain) continued until at least 1985 (Table 1). There are no significant natural sources of PCBs. There are 209 congeners of PCBs (Fig. 1), although the majority of PCBs found in wildlife tissues consist of only 30–40 congeners.

Their use has been highest in areas of high industrial density, such as foundries, power generation, manufacturing centres, etc. Though often used in "closed systems" such that they minimize environmental releases, PCBs were also used in "open systems", such as in paints, hydraulic fluids, caulking and other products that

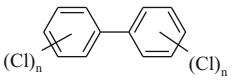
Table 1 Annual production of polychlorinated biphenyls (PCBs) in tons from 1954 to 1984, by country. Data based on [3]

Year	USA	France	FR Germany	Italy	Spain	UK	Japan	Russia
1954							450	
1955							1,000	
1956							1,100	
1957	14,651						1,900	
1958	11,821						1,950	
1959	14,202						2,750	
1960	15,973						3,600	
1961	17,027						4,850	
1962	17,256						4,800	
1963	17,296						4,000	
1964	20,352						5,650	
1965	23,494						6,600	
1966	26,797						9,700	
1967	28,334						9,850	
1968	29,536						11,300	
1969	30,479						17,000	
1970	33,140						17,000	
1971	15,559						24,450	
1972	11,978						14,900	
1973	17,119	9,674	9,649	2,519		4,067	3,200	
1974	15,605	9,541	8,374		1,935	4,818	0	
1975		7,182	7,328	1,868	2,500	3,274	0	
1976		7,190	6,610	1,933	2,100	3,013	0	
1977		7,640	5,680	2,343	1,700	2,830	0	
1978	0	7,916	7,640	1,767	1,600	0	0	
1979	0		7,280	1,414	1,400	0	0	
1980	0	6,577	7,309	1,479	1,200	0	0	
1981	0		4,778			0	0	
1982	0		3,734			0	0	
1983	0		4,335			0	0	
1981–1984	0	14,983	13,300	4,388	3,296	0	0	
Total	360,619	70,703	86,017	17,711	15,731	18,002	146,050	180,000

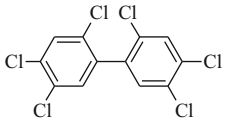
had a strong potential for the PCBs to be released into the environment. Releases of PCBs continued (and continues) long after the production has stopped, as they leach from open systems as the products remain in use, or continue to degrade. For example, although production of PCBs or sales of products containing PCBs stopped in 1977 in North America (Table 1), transformers and capacitors containing PCBs continue to be used in North America at the time of this writing (2015). Furthermore, soils and sediment in particular act as reservoirs of PCBs (and other POPs), which can be released into the environment through erosion or volatilization.



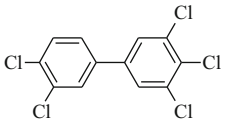
2,3,7,8-tetrachlorodibenzo-*p*-dioxin
(i.e. TCDD)
Log K_{OW} 6.42



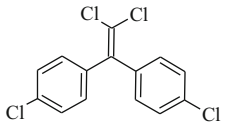
Polychlorinated Biphenyl (generic)
Log K_{OW} 4.58 - 8.27



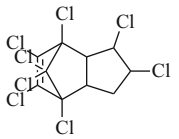
2,2′,4,4',5,5′ -hexachlorobiphenyl
(i.e. PCB 153)
Log K_{OW} 6.89



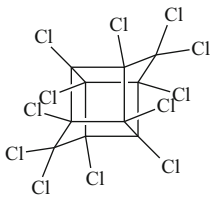
3,3',4,4',5-pentachlorobiphenyl
(i.e. PCB 126)
Log K_{OW} 7.75



1,1'-(2,2-dichloro-1,1-ethenediyl)bis(4-chlorobenzene)
(i.e. *p*,*p*′-DDE)
Log K_{OW} 6.51



1,3,4,7,8,9,10,10-octachlorotricyclo[5.2.1.02,6]dec-8-ene
(i.e. Chlordane)
Log K_{OW} 6.22



1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1H-1,3,4-(methanetriyl)cyclobuta[cd]pentalene
(i.e. Mirex)
Log K_{OW} 6.89

Fig. 1 Example of persistent organic pollutants and their respective log K_{OW}s; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, general structure of polychlorinated biphenyls (PCBs), PCBs 153 and 126 (IUPAC), *p*,*p*′-DDE, chlordane and mirex

Approximately 1.1 million tonnes to 1.8 million tonnes have been produced, of which 0.2–0.4 million tonnes are bioavailable [4, 5]. Although PCBs are virtually always considered to be "legacy" compounds, in that they are no longer being produced, some PCBs are currently being formed as a by-product in the manufacture of other industrial or commercial compounds. For example, due to the manufacture of diarylide yellow pigment, approximately 1.5 t of PCB 11 was inadvertently produced in 2006 [6], which is to date the only known significant source of PCB11 [7].

Although PCBs do travel long distances via airborne transport and other processes [8], generally exposure and thus body burdens are highest in areas near their sites of manufacturer or high use or at sites of accidental or purposeful releases [9–11].

2.2 Organochlorine Pesticides

Unlike PCBs, pesticides are virtually exclusively, by their very nature and purpose, released directly into the environment, often over very large areas. Also unlike PCBs, legacy organochlorine pesticides include a number of structurally unrelated chemical classes (Fig. 1). With some exceptions, concentrations in environmental matrices, including body burdens in wildlife, tend to be highest in areas of high historical agricultural activity. Given that, however, much of the terrestrial environments are agricultural based, a significant proportion of the Earth's surface, excluding Antarctica and the Arctic, had historical or current use of organochlorine pesticides, such as DDT and chlordane. For example, DDT and their metabolites have been found in high concentrations in wildlife in areas that had high historical use. DDT is the most famous of the organochlorine pesticides, and approximately 1.8 million tonnes have been produced since the 1940s [12]. Although the use of DDT was banned in the USA and Canada in 1972, its use continued in other countries primarily for the control of mosquito-transmitted malaria, although India is currently the only country that manufactures DDT and is in turn the main user of DDT [13].

Technical chlordane is a mixture of varying proportions of *cis*-chlordane, *trans*-chlordane and heptachlor, as well as other compounds at much lower concentrations, some of which are racemic in that they consist of mixtures of enantiomers of each chiral compound [14]. Mirex is an insecticide derivative of cyclopentadiene that was produced primarily to kill fire ants but also had some minor uses as a flame retardant [15] and as a visual enhancer in fireworks. Mirex was banned as a pesticide in 1976 in the USA, although in some other countries it wasn't banned until much later, such as Thailand in 1995. Dieldrin is another cyclopentadiene derivative, and in most "western" countries its use was banned in the mid-1980s, and generally the use of dieldrin was banned in the late 1980s or early 1990s elsewhere, such as in Southeast Asia.

2.3 *Polychlorinated Dibenzo*-p-*Dioxins and Furans*

Unlike either PCBs or organochlorine pesticides, polychlorinated dibenzo-*p*-dioxins and furans (Fig. 1; PCDD/Fs) were not commercial products but rather were by-products of the manufacture of chlorine-containing organic compounds, such as PCBs or pesticides, or through the incomplete combustion of chlorine-containing organic compounds, such as plastics. Some of the main sources are from stationary sources such as waste incineration and ferrous and non-ferrous metal production and processing power plants but also diffuse sources, such as traffic emissions [16]. Given that PCDD/Fs are sometimes associated with other products such as PCBs or pesticides, concentrations of PCDD/Fs are frequently highest in areas that have high concentrations of PCB or pesticides known to have PCDD/F contaminations [17]. Mixtures of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in herbicide products, popularized by names such as Agents Orange, Pink, and Purple, have been heavily used in some areas, until they were removed from production due to TCDD contamination in the manufacturing process of 2,4,5-T [18]. The so-called rainbow herbicides were made famous due to widespread 2,3,7,8-TCDD contamination in the herbicides in Southeast Asia during the period of American involvement in the Vietnam War, from 1961 to 1971. These herbicide mixtures were sprayed over large areas so as to destroy plant-based agriculture in areas of communist control and to act as defoliants so as to deny opposing forces foliage as cover during military operations. Similarly, TCDD contamination has been a problem on American or allied military bases.

Despite being an undesired by-product of contamination in commercial products, given the chemical similarity to PCBs – both being chlorinated di-phenolic compounds – their fate and distribution are similar to PCBs. One of the important differences, however, is the much greater toxicity compared to PCBs or other POPs [19]. There are 75 possible congeners of dioxins and 135 congeners of furans, of which 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is generally the most toxic.

2.4 *Dioxin Toxic Equivalents*

Although not a chemical class per se, a brief discussion of dioxin toxic equivalents (TEQs) is warranted. Many PCDD/Fs mediate their toxicity through binding with the aryl hydrocarbon (Ah) receptor, which is mostly associated with the mediation of genes (e.g. cytochrome P450 1A, 1B and 2S) associated with the metabolism of xenobiotics (see Sect. 4.1). However, the Ah receptor affects other pathways that alter development, cell cycle regulation, hypoxia and cold tolerance and stress responses [20]. Many PCBs, particularly non-*ortho* and mono-*ortho* PCBs (77, 126 and 169 and 105, 118 and 189, respectively), as well as PAHs and other

aromatic compounds, will also interact with the AH receptor and thus exhibit dioxin-like activity [19, 21–23]. Generally, 2,3,7,8-TCDD is the most toxic dioxin, but the toxicity of approximate isostereomers of 2,3,7,8-TCDD is predictable depending on the relatively binding affinity to the Ah receptor by the different congeners [24]. The predictable toxicity of this family of contaminants based upon their binding affinity to the Ah receptor allows for the calculation of toxic equivalency factors (TEFs), which express the toxicity of each compound relative to that of 2,3,7,8-TCDD [25]. Multiplying the concentrations of each compound with the respective TEFs gives the toxic equivalents (TEQs) of 2,3,7,8-TCDD [24], at least under the assumption that the effects of individual dioxin-like compounds are additive. The TEQ approach can then be used to assess the toxicity of complex mixtures, at least for dioxin-like compounds, and TEQs are frequently used in regulatory actions and risk assessment [26].

3 Exposure

Wildlife is exposed to organic contaminants that were released into the environment through historic or current sources, such as through municipal or industrial effluents, airborne emissions, leaching from the use of POP-containing products, pesticide use, open use of industrial chemicals or processes, emissions from landfills or similar sources. Wildlife may be exposed to POPs through dermal exposure, diet and inhalation or through maternal transfer. The relative importance of the different avenues of exposure depends on the physical-chemical properties of the compounds of interest, metabolism, physiological condition of the animal, dietary habits and other factors. Although there are multiple potential sources of exposure, generally, POP exposure in vertebrates is considered to be primarily through diet. Nonetheless, non-dietary routes of exposure to POPs have been important in certain circumstances, which will also be discussed below.

3.1 Partitioning of POPs

3.1.1 Role of Lipids in Fate of POPs

On a wet weight basis, concentrations of lipophilic compounds may vary considerably amongst tissues. Concentrations of PCBs, for example, were 910 × higher in liver than in plasma of bald eagles [27]. However, according to the equilibrium lipid-partitioning theory, POPs should be sequestered equally amongst tissues, relative to the lipid content of each tissue [28]. Lipids are generally the main component driving the movement, distribution and sequestering of lipophilic

compounds, and thus POPs are associated with lipid membranes and lipid stores [29]. All other factors being equal, concentrations of lipophilic contaminants, therefore, will be proportional to the concentration of lipids in each tissue. Thus, concentrations are generally highest in adipose and other lipid-rich tissues (eggs, liver, etc.) and lowest in plasma and muscle [28, 30].

More detailed examination of lipid partitioning reveals that bioaccumulation varies amongst lipid classes [31, 32]. Lipid classes, such as phospholipids (relatively polar), triacylglycerols (non-polar) in storage lipids and glycolipids, triglycerides and free fatty acids (neutral polarity) have different properties and therefore tendency to sequester POPs. In blood, POPs are associated with lipoproteins and proteins. Compared to subcutaneous fat and organ lipids, the lipid fraction of plasma is more polar and contains relatively fewer non-polar lipids [33]. The lactational transfer of POPs to young mammals has been associated to some degree with the association of POPs to proteins like albumin in the plasma, which has been seen in both mice and humans [34, 35]. Given that the nature of lipids differs amongst tissues, expressing contaminant burdens in plasma on a lipid basis may overestimate burdens compared to other tissues [36].

Regardless, generally environmental scientists measure total lipids as an index of lipid partitioning, given the costs of measuring different lipid components. There are also a number of exceptions to the sequestering of POPs to lipids. Although hydroxylated PCBs (HO-PCBs) are also lipophilic, some HO-PCBs are reversibly bound to proteins, such as transthyretin in the blood, and thus their accumulation is not necessarily dependent on lipid content [37], but instead a few HO-PCBs make a disproportionately high proportion of the total hydroxylated PCB burden.

As a tool to estimate bioaccumulation based upon lipophilicity, octanol-water partition coefficients (log K_{OW}) are often measured or, failing availability of empirical measurements, are sometimes estimated using various models. Generally, contaminants are classified as potentially bioaccumulative if they are extremely lipophilic, where the solubility in lipids is about ~100,000 × of that in water. Hence, lipophilic compounds with log $K_{OW} \geq 5$ biomagnify in aquatic ecosystem [38, 39], and those with log $K_{OW} < 5$ do not [40], and with a reduced bioaccumulation potential with a log $K_{OW} \geq 7$ due to steric interference or low intestine absorption. However, in terrestrial environments, this relationship may not hold. Vertebrates with lungs (and thus air breathing), like their aquatic cousins, also bioaccumulate compounds that have a log $K_{OW} \geq 5$, but they can also bioaccumulate compounds with a log $K_{OW} < 5$ and a high octanol-air partition coefficient (K_{OA}). Compounds with a $K_{OW} > 2$ and $K_{OA} > 6$ are potentially bioaccumulative in animals with lungs, as these compounds would have a slow rate of partitioning from the lipid-rich lung to air, resulting in reduced elimination rates [40]. For example, the pesticides dicofol, ß-endosulfan, ß-HCH and trifluralin do not biomagnify in fish due to their low lipid solubility (K_{OW} 3.5–4.4), but they can biomagnify in air-breathing vertebrates [40].

3.2 Maternal Transfer

3.2.1 Viviparity

Placental viviparous species, which is the sole domain of placental mammals, can transfer POP burdens from the female to the young not only during placental development but also through feeding the young with milk. The importance of maternal transfer should not be understated in terms of the exposure of the young; many studies have found that juvenile placental mammals often receive larger doses of PCDD/Fs or PCBs through feeding on milk than they do from prenatal exposure during placental development. Chen et al. [41] found that following an oral gavage of pregnant rats with six PCDD/Fs, including 2,3,7,8,-TCDD/F, and the non-*ortho* PCBs 77, 126 and 169, the offspring received 7–28% of their doses lactationally and only 0.5–3% than through the placenta. Fasting by mothers can exacerbate the dietary exposure of young to POPs from milk. Polar bears fast during some of the period of lactation, and although the trend in body burdens of the females was not consistent – DDT and HCH declined during fasting, whereas chlordane and PCBs increased – the ratio of plasma/adipose tissue and milk/adipose tissue OC concentrations did not change during the fast, which indicates that the POPs were probably at steady state amongst the different tissues [42]. However, the concentrations of chlordane and PCBs in the milk increased during the fast, and consequently, the whole-body concentrations of these POPs increased in the nursing cubs.

Debier et al. [36] examined the maternal transfer of PCBs from female grey seals (*Halichoerus grypus*) to their young through their milk. Although the PCB content of the milk was initially constant during early lactation (0.31 μg/g) during a period of increasing lipid content, during late lactation the PCB content (0.67 μg/g) increased even though the lipid content at that point remained constant. At birth, pups had significantly higher PCB burdens (11.9 ng/ml) in serum compared to the mother's serum (6.7 ng/ml), indicating that the females preferentially sequestered PCBs to the pups during placental development. Mean PCB concentrations in pup serum were 11.86 and 27.89 ng/ml during the period of early and late lactation, respectively, and those of the females were 6.69 and 12.18, respectively; hence, body burdens of both pups and mothers increased during the lactation period [36].

3.2.2 Oviparity

Oviparous species, such as all birds, most reptiles, most amphibians and the rare monotreme mammals, will pass POPs to their eggs through maternal transfer. The main source of POP exposure to the developing embryo is the direct transfer of the contaminant burden from the female to the eggs through the reallocation of the female's lipid stores. The deposition of lipids and proteins in the developing egg (along with the POPs associated with the lipids and proteins) is driven by both

biological processes and chemical properties of the contaminants. The energy consumed by the egg formation may be derived from older body reserves of the female, energy intake by the female during the period of egg formation or a combination of both [43]. POPs such as PCBs, organochlorine pesticides and PCDD/Fs sequester primarily in lipids, which are incorporated into the egg – primarily the lipid-rich yolk – during oogenesis. During oogenesis, lipids are mobilized from the female's fat stores, which are used during yolk proteins synthesis in the liver, and are then transported through the blood to the developing egg [44, 45].

Nonetheless, factors other than the passive sequestering of POPs within lipid reserves must govern the maternal transfer of POPs from the female to the eggs, as the degree and relative rate of transfer of individual compounds into the developing egg are not consistent with lipid solubility only. Van den Steen et al. [46] found that the maternal transfer of PCBs, organochlorine pesticides and polybrominated diphenyl ethers (PBDEs) was biased towards compounds with higher lipid solubility and biological persistence in blue tits (*Cyanistes caeruleus*). Verreault et al. [47] reported that maternal body selectively retained the more highly chlorinated PCB congeners, and hence lipid soluble congeners, instead of being transferred to the developing egg. Similarly, the eggs of snapping turtle (*Chelydra serpentina*) favoured the lower chlorinated PCB congeners compared to maternal liver or adipose fat [48]. Some of the differences in observations, however, may be due to the selection of tissues for the assessment of maternal burden, as Zheng et al. [49] demonstrated from laying chickens (*Gallus domesticus*) fed on a farm surrounded by e-waste recycling workshops in Guangdong Province, China. The most recalcitrant PCB congeners, such as 138, 153 and 180, had the highest rate of transfer from the females to the eggs, relative to the burdens in the females muscle or liver. Conversely, there was no bias related to recalcitrancy or lipid solubility when the burdens in the eggs were compared to visceral fat. Hence, they found that chickens used primarily visceral fat for the energy source during egg formation, which was the primary source of PCBs deposited in the eggs [49]. Although the structure of the eggs in reptiles differs significantly from those of birds, the process of maternal transfer is believed to be largely the same. Bishop et al. [50] found that contaminants deposited in snapping turtle eggs were primarily from recent diet instead of body adipose stores. Similar to the reduced body burden of mammalian females due to the loads transported to their young though lactation, the lower contaminant burdens in female turtles relative to males have been attributed to the loss of contaminants during egg production [50, 51].

3.3 Eggshell Exposure

Developing embryos may also get exposed to POPs through absorption of mobile contaminants through their eggshells. For most avian species, direct exposure of the eggs to contaminants from the nesting material or from the local soils is likely

minimal. Avian eggs tend to be relatively impermeable to chemicals, except perhaps those in the gaseous phase, given the air exchange between the embryo and the surrounding air. An exception are topical applications of oil-based mixtures to eggs, usually deliberate for control purposes, which can kill the embryos by asphyxiation as the oil prevents air exchange (e.g. [52]). However, reptilian species tend to have eggs that are generally more permeable than avian eggs. Developing embryos of reptilian species can be exposed to organic contaminants from the nesting substrate, although it is not known whether exposure is generally from contaminants that are dissolved in free water in the nesting substrate or from the gaseous phase through air exchange across the eggshell. Crocodilian eggshells have large-diameter pores, whereas the eggshells of many chelonians have loose texture and poorly organized crystallites. These structures allow substantial air and water exchange during development [53, 54]. Amphibian eggs are generally considered permeable to contaminants, but given that extremely few lay eggs terrestrially, they are not considered here.

Both lizard and turtle eggs have been shown to absorb metals from contaminated soils [55, 56]. Following the dosing of a nesting substrate of Spanish moss and vermiculite with aldrin, *p*,*p*′-DDT, dieldrin, endrin, heptachlor and lindane, incubated bullsnake (*Pituophis melanoleucus*) eggs absorbed low concentrations of five of the pesticides and absorbed about 20% lindane, but only between 0% and 5% of endrin, aldrin, heptachlor, dieldrin and DDT [57]. Absorption of the pesticides into the eggs increased with lower lipid solubility of the pesticides. Turtle eggs exposed to 10 herbicides, insecticides and fungicides through treated soil readily absorbed most pesticides, except for chlorothalonil [58]. Absorption increased over time, and six of the ten pesticides were detected in the eggs after only 24 h of exposure and nine pesticides detected after 8 days of exposure, to a 1.92 kg/ha application of the active ingredients, which yielded an average of about 1.2 μg/g (dw) in soil.

3.4 Diet

Dietary intake is the most commonly study route of exposure of contaminant exposure in animals missing gills, and many POPs, such as methylmercury and PCBs, bioaccumulate and biomagnify through food chains [59, 60]. Although not necessarily true with animals that have gills, diet can be the dominant route of exposure if the compounds in question are persistent and highly lipophilic. It is a well-known phenomenon that, all other things being equal, POP body burdens increase with trophic level, at least within ecosystems. The concept of biomagnification largely started in the 1960s, where, for example, Woodwell et al. [61] determined that DDT concentrations increased from 0.04 μg/g (ww) in plankton to 75 μg/g in ring-billed gulls and that higher trophic level animals (e.g. tertiary predators) had higher concentrations than lower trophic level animals.

As stated earlier, generally, contaminants are compounds classified as bioaccumulative if their log K_{OW} is between 5 and ~7–8. However, the K_{OW} only

predicts the passive partitioning of the contaminant between water and lipids, but bioaccumulation is also driven by assimilation efficiency, capacity and rate of enzymatic metabolisms and capacity to excrete the chemicals. Highly lipophilic compounds are sequestered in lipids in the body burden of the prey, and after ingestion the chemicals are absorbed through the digestive tract, transported by the blood throughout the body and deposited into lipid-rich tissues. Typically, animals at higher trophic positions have the greatest potential for bioaccumulation of POPs [62]. However, bioaccumulation of compounds is not proportional to trophic position for contaminants that are less recalcitrant, due to lower lipid solubility or ease of metabolism and thus excretion [28, 63].

Although older studies have determined trophic level through studies of gut contents or behavioural observations of dietary choices, more recent studies have used ecological tracers, such as stable isotopes and fatty acids, to investigate food web trophodynamics. The isotope fractionation of stable nitrogen isotopes ($^{15}N/^{14}N$, expressed as $\delta^{15}N$) in biotic tissue can estimate the organisms' trophic position, as the ratio increases in a predictable fashion from one trophic level to the next [64, 65]. During the process whereby amine groups (R–CO–NR′R″) are added or removed from proteins, the heavier ^{15}N isotope is enriched relative to the lighter ^{14}N isotope by 3–4‰ per trophic level [64, 65]. Changes in the food web may affect the trophic position of predators, which may affect estimates of the bioavailability of POPs in the environment [66] and trophic magnification factors (TMF). For example, following changes in the Arctic food web, from 1993 to 2013 the trophic position of thick-billed murres (*Uria lomvia*) declined, which affected the apparent rates of decline of POPs in murre eggs [67]. Similar declines in trophic position of herring gulls (*Larus argentatus*) in the Great Lakes resulted in a reduced rate of decline in egg burdens of POPs, when the rates of decline were adjusted for the change in trophic position [66].

Chaiyarat et al. [68] examined organochlorine pesticide residues in birds from three different trophic groups (carnivorous, omnivorous and insectivorous) from the Boraphet wetland (Thailand), which is a floodplain wetland surrounded by extensive agricultural land. Further, these pesticides (DDT, chlordane, aldrin, amongst others) were banned for use in 1983, meaning that body burdens of birds would reflect historical and not recent use. Although burdens of pesticides differed amongst species within each trophic group, the largest differences were amongst trophic groups. The carnivorous species [little cormorant (*Phalacrocorax niger*), yellow bittern (*Ixobrychus sinensis*) and long-tailed shrike (*Lanius schach*)] averaged 95.8, 76.1 and 30.9 ng/g (ww) of total DDT, total endosulfan and total HCH, respectively, whereas the omnivorous species [pheasant-tailed jacana (*Hydrophasianus chirurgus*), Asian pied starling (*Sturnus contra*) and purple swamphen (*Porphyrio porphyrio*)] averaged 2.8, 0.5 and 18.7 ng/g. The insectivorous species had only 0.1 ng/g total DDT, 5.4 ng/g endosulfan and 9.8 ng/g of HCH [68].

3.5 Biomagnification

Although the majority of biomagnification studies focus on marine or freshwater environments, some pertinent terrestrial studies have been conducted. Bioaccumulation of PBDEs was examined in a terrestrial ecosystem near Antwerp (Belgium), with both avian predatory species (buzzard [*Buteo buteo*], sparrowhawk [*Accipiter nisus*]) and a fox [*Vulpes vulpes*] species [69]. Although there appeared to be significant biomagnification in the primarily predatory birds, with biomagnification factors (BMFs) of PBDEs ranging between 2 and 34, foxes had body burdens of PBDEs that were lower than their rodent prey, which was suggested to be due to the high metabolic capacity of the fox [69]. It should be noted that, although all are predatory species, foxes have a much more omnivorous diet than the two predatory birds, which may have reduced the dietary exposure of the fox relative to its avian counterparts. Blankenship et al. [70] examined BMFs in the terrestrial ecosystem at the Kalamazoo River Area of Concern, which was impacted by the discharge of PCB laden waste from the recycling and processing of carbonless copy paper, between 1957 and 1971 [71]. The BMFs of total PCBs from plant to small mammals were near unity (0.65–1.1), BMFs from terrestrial invertebrates to passerine birds ranged from 3.1 to 35, and from small mammals to great horned owls (*Bubo virginianus*), the BMFs ranged from 13 to 40. Despite the increase in body burdens at the higher trophic levels, the relative potency of the PCBs, as expressed as the mg of TEQs per mass of PCBs, decreased up the food chain [70], putatively due to extensive weathering of PCBs from the initial Aroclor 1242 source. When tracking the individual congeners from the lower food base to the predators, the congener profile was enriched in more recalcitrant congeners, such as 153, 180, 138, 118 and 99.

The BMFs of a number of legacy and emerging POPs, including DDTs, PCBs, PBDEs, hexabromocyclododecanes (HBCDs) and dechlorane plus (DP), were determined in two food chains from a metropolis region in Beijing, China [72]. The first consisted of the common kestrel (*Falco tinnunculus*) and their frequent prey the Eurasian tree sparrow (*Passer montanus*) and the second the eagle owl (*Bubo bubo*) and little owl (*Athene noctua*) and their frequent prey the brown rat (*Rattus norvegicus*). Overall, the BMFs varied amongst compound as DDE $>$ PCBs $>$ PBDEs; the BMFs for PCB congeners ranged between 1.5 and 20 (mean 6.6) for kestrels vs. sparrows and between 5.1 and 50 (mean 24.4) for owls and rats, whereas BMFs for PBDE congeners ranged between 0.26 and 6.9 (mean 1.9) for kestrels vs. sparrows and between 2.2 and 50 (mean 15.4) for owls and rats [72]. In general, the log BMF decreased with increasing log K_{OW}, at least within the range of values in this study (log K_{OW} 6–10).

BMFs were calculated for fish and osprey eggs from Willamette River (Oregon, US), from 1993 and 2001 [73]. Ospreys are obligate piscivores, thus simplifying the estimates of BMFs, by reducing the likelihood of misspecifying the predator-prey relationship. Higher chlorinated dioxins had the highest lipid-adjusted BMFs of the compounds examined, with BMFs ranging between 24 (for 2,3,7,8-TCDD) and

290 (for 1,2,3,6,7,8-HCDD). Interestingly, the BMFs were much lower for furans compared to dioxins, with BMFs between 0.46 and 16 for PCDFs [73]. The BMFs for PCBs were about 13. Grove et al. [74] summarized BMFs of osprey in their literature review and concluded that, not surprisingly, BMF estimates differed amongst contaminant classes in relation to their chemical properties and that the BMFs for PCB ranged between 10 and 20, and the BMFs for dieldrin, heptachlor epoxide, DDE and OCDD were 6.7, 25, 87 and 174, respectively.

Selective metabolism and elimination can have marked effects on the body burdens of POPs. Even though the concentrations of POPs may increase at higher trophic levels, many of the more easily eliminated compounds may not biomagnify or sometimes are lost entirely. Organochlorine pesticides were tracked through three trophic levels in the Arctic by Elkin [75], from lichen to the herbivorous caribou and finally the wolf top predator. Although the concentrations of most of the pesticides and PCB congeners increased with trophic level, the congener pattern shifted up the food chain. Lower chlorinated PCBs (e.g. PCB 66/95, 70, 101 and 52 as the top four) were dominant in lichen, but these congeners essentially disappeared from the burdens in the wolves (PCBs 180, 170/190, 153 and 194 as the top four), whereas the congener profile in the caribou was intermediate between the two. Even when comparing similar food webs in different regions, the pattern of bioaccumulation may vary. BAFs that were measured in Arctic marine animals tended to be lower than predicted based on the log K_{OW} of the contaminants, yet at the Barents Sea and White Sea the BAFs were markedly higher than those predicted [62].

3.6 Inhalation

Although diet is the main route of exposure, due to the tendency for POPs to sequester to lipids, airborne exposure can, for some compounds or situations, be an important source of exposure or elimination. Despite their (generally) low volatility, many POPS can be found in both gaseous and particulate phases in the atmosphere (e.g. [76–78]). Compounds like PCBs and organochlorine pesticides can be transported thousands of kilometres from their source, either through (1) aqueous transport of chemicals in the dissolved state or by being bound to suspended solids or (2) airborne transportation, as gaseous phase or particulate phase. Even compounds that are not traditionally considered to have a significant inhalation exposure route, such as PCBs, can have appreciable inhalation exposure [79]. Hu et al. [80] found that PCB 11, a congener not generally considered to have a significant legacy or historical source, can be bioavailable to humans by airborne exposure at sufficient concentrations to form measureable levels of hydroxylated metabolites.

A crucial difference between aquatic respiration and air-breathing animals is that while their main route of exposure to POPs is likely to be the same (e.g. through diet), exposure and elimination through respiration differs. Air-breathing animals

can inhale airborne POPs in either the gaseous or particulate phase and eliminate the same through exhalation (or through the "normal" route of excretion in urine or faeces), whereas animals with gills may absorb POPs dissolved in the water. Thus, bioaccumulation of chemicals through respiration in air-breathing vertebrates will be dictated by, amongst other factors, the partitioning of the chemical between air and lipids whereas, for aquatic animals, between water and lipids. As stated earlier, using QSAR modelling, Gobas et al. [39] found that chemicals with a log K_{OW} as low 2 can bioaccumulate in terrestrial (air-breathing) wildlife, given situations where the log $K_{OA} > 5$ or 6. Similarly, in situations where a compound is lipophilic yet has high affinity to air, the lungs may be a major route for elimination, with some exceptions. Even though it was found that there can be appreciable airborne exposure to PCB 11, the parental compound and metabolites of PCB 11 were eliminated rapidly through excretion of urine and faeces [80].

3.7 Aquatic vs. Terrestrial Biomagnification

There has been some argument that biomagnification for persistent lipophilic compounds can be higher in terrestrial versus aquatic food chains, such as by Gobas et al. [39]. As an example, biomagnification factors for terrestrial mammals, such as wolves and birds, are generally an order of magnitude higher than for aquatic organisms, such as fish and aquatic invertebrates [39]. Aquatic feeding birds accumulated BDE-47 more readily than birds that fed primarily from the terrestrial ecosystem, which instead had greater accumulation of BDE- 153 and higher brominated congeners [81, 82]. Furthermore, the fugacity of many POPs is very high from water to aquatic organisms, leading to bioconcentration or direct accumulation of POPs through passive partitioning, which many scientists mistake for biomagnification [83]. Tetrachlorobenzene, for example, is primarily accumulated in trout (*Salmo trutta*) through the gills rather than from diet [32]. Similarly, Paterson et al. [84] showed that concentrations of PCBs did not generally increase through the food chain in a lake and that much of the differences in PCB content of different fish species were largely due to differences in lipid content.

Gray [83] suggested that some type of lipid normalization for concentrations of POPs should be used, so as not to "...naïvely interpret results as evidence for biomagnification...", rather than bioconcentration, which is occurring. However, the BMFs of POPs in marine mammals are generally an order of magnitude greater than non-mammalian aquatic organisms [39] and are consistent with terrestrial animals [85]. Although perhaps a minor criticism, statements regarding the relatively greater tendency for biomagnification to occur in terrestrial vs. aquatic ecosystems may be somewhat misplaced. Based upon the arguments put forth above, the differences in biomagnification of POPs could be explained primarily by the differences in respiration in lungs vs. gills and hence the similarity in biomagnification between mammals from terrestrial and aquatic environments. While in general the log K_{OW} is seen as one of the better predictors of

bioaccumulation potential, the log K_{OA} is sometimes a better predictor of bioaccumulation in terrestrial ecosystems.

4 Metabolism

Another critical factor to bioaccumulation, beside exposure (dietary or otherwise), is elimination, which is heavily regulated by xenobiotic metabolism; at least for species with significant capacity to metabolize POPs, such as avian and mammalian species. Hepatic metabolism may be the rate-limiting step for the elimination of body burdens of POPs [86]. Following metabolism, the metabolites are subsequently eliminated through the urine or faeces. The capacity to eliminate chemicals is probably, at least loosely, related to the field metabolic rate (FMR). The FMR for birds is generally higher than mammals, albeit with much overlap between those two taxonomic groups, and both are much higher than the FMR for reptiles [87]. At the very least, animals with higher metabolic rates would have greater energetic demands and thus in turn would have a higher daily food requirement [88], leading to greater dietary exposure to POPs. Negative correlations have been found between basal metabolic rates and body burdens of sum PCBs, DDE and other organochlorine pesticides in glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic [89].

4.1 Phase I and Phase II

The cytochrome P450 family of proteins are a key component of the ability of wildlife to metabolize both endogenous and exogenous organic compounds (including POPs), by catalyzing the addition of oxygen to the carbon skeleton. Although the cytochrome P450 family provides numerous functions, such as steroidogenesis or fatty acid metabolism, one of the main purposes is detoxifying and hence eliminating xenobiotics, which includes POPs. Phase I metabolism by cytochrome P450 enzymes, particularly the subfamilies 1, 2 and 3, of chlorinated POPs occurs by the hydroxylation or oxidation of the substrate through a number of different potential mechanisms [21, 90]: hydroxylation at an unsubstituted position (particularly vicinal hydrogens), hydroxylation with an NIH shift with chlorine [91], hydroxylation with the elimination of chlorine or oxidation through the formation of epoxides ([90]; Fig. 2). Phase I increases the rate of elimination of POPs, as the hydroxylation increases the water solubility and thus increases the rate of elimination through the kidney or intestines and increases the rate of Phase II metabolism. Phase II metabolism entails further metabolism of the Phase I metabolite, of either the hydroxylated or epoxide forms, by conjugating them with other functional groups, such as glutathione, glucuronic acid or methylsulfonyl, which results in

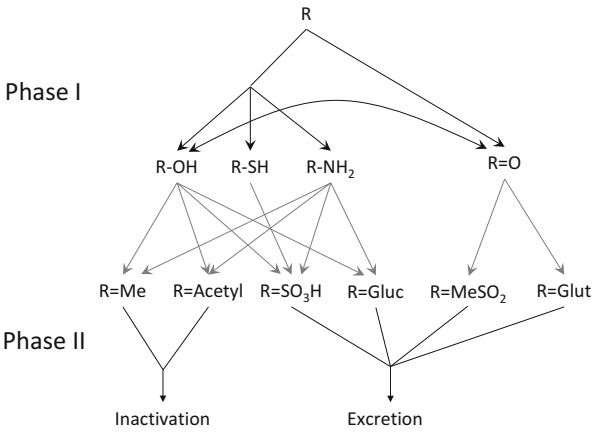


Fig. 2 General pathways of Phase I metabolism (oxidation, reduction or hydrolysis) by cytochrome P450 and Phase II metabolism, through conjugation by methylation (R-Me), sulphation ($R\text{-}SO_3H$), acetylation (R-Acet), glucuronidation (R-Gluc) and glutathione conjugation (R-Glut). Metabolism of persistent organic pollutants will follow only a subset of these pathways

much more water-soluble metabolites that are (putatively) excreted at a much faster rate than their parental compounds (Fig. 2) [92, 93].

4.1.1 Taxonomic Variation in Cytochrome P450

The cytochrome P450 superfamily of proteins are found in virtually all forms of life, but both the amino acid sequence and activity differ, sometimes considerably, amongst taxonomic groups [21, 94–97]. There are 265 different families of cytochrome P450 enzymes, and the specific isoforms vary amongst taxonomic groups [98], which results in the marked difference in the ability of different taxonomic groups to metabolize POPs. The genomic region encompassing the cytochrome P450 region has a very high rate of background mutation and hence has a high evolutionary rate of change [99].

Turtles have at least two microsomal and hepatic cytochrome P450 1A forms and likely have multiple forms of other cytochrome P450 families, such as 2B and 3A, and, given the relatively poor state of research on reptilian cytochrome families, other undescribed forms of cytochrome P450 proteins [100]. Nonetheless, cytochrome P450 activity has been verified in reptiles, and both snakes and alligators have protein expression consistent with cytochrome P450 1A [101–103], and further alligators also have cytochrome P450 2B forms [104]. Alligators that were exposed to 3-methylcholanthrene exhibited a response that was similar to those of mammals similarly exposed [103], albeit with a lower level of induction. The rates of induction of cytochrome P450 1A by xenobiotics, and hence the rate of metabolism of those xenobiotics, in reptiles are substantially slower than those reported for mammals and birds [102, 104]. Although turtles metabolize PCB 77, for example, at similar rates of the least metabolically capable avian species, the rate of metabolism is less than typical birds and certainly less than placental mammals [105]. In one example, following exposure to brominated flame retardants, snapping turtles (*Chelydra serpentina*) failed to exhibit any significant

metabolic ability, whereas red-eared sliders (*Trachemys scripta elegans*) exhibited some debromination of PBDE 99 to 47 [106]. American alligators from three sites from Lake Okeechobee and the central Everglades, each with varying exposure to historical use of OC pesticides from muck farming operations, demonstrated significant cytochrome P450 1A induction, but no induction of cytochrome P450 2B or Phase II metabolism [101], although the rate of cytochrome P450 1A activity did not follow a typical dose-response relationship.

Richardson and Schlenk [107] measured the rates of biotransformation of two PCB congeners in hepatic microsomes from four species of sea turtles, green (*Chelonia mydas*), olive ridley (*Lepidochelys olivacea*), loggerhead (*Caretta caretta*) and hawksbill (*Eretmochelys imbricata*). There was no observed hydroxylation of PCB 77, which is normally putatively metabolized by cytochrome P450 1A in the turtle microsomes. PCB 52, putatively metabolized by cytochrome P450 2B, was metabolized at rates ranging $<$ 0.5–53 pmol/min/mg protein. Although specific cytochrome P450 proteins were not identified, cytochrome P450 family 2-like and to a lesser degree family 3-like hepatic proteins appeared to be responsible for the hydroxylation in sea turtles [107].

In general, Phase I metabolism is strongest in birds and mammals compared to other taxonomic groups, although there is considerable overlap in potency. Using cytochrome P450 1A1, 1A2 and 1B1 of both humans and Baikal seals (*Pusa sibirica*) embedded in yeast [108], the catalytic activities of the seal's cytochrome P450 1A1 were lower than that of humans, and 1A2 were minimal, whereas the seal's 2B1 activities were higher than that of human 1B1. At least some of the differences between seal and human cytochrome P450 1A2 activities appeared to be due to differences in the amino acid sequence in the I helix region.

Boon et al. [109] evaluated the relative potencies of hepatic microsomes of three mammals (harbour seal [*Phoca vitulina*], white-beaked dolphin [*Lagenorhynchus albirostris*] and sperm whale [*Physeter microcephalus*]) and one avian species (Laysan albatross [*Diomedea immutabilis*]), following hepatic in vitro exposure to a technical toxaphene mixture. The capacity to metabolize toxaphene through Phase I biotransformation was, in order from highest to lowest, seal $>>$ dolphin $\approx$ albatross $>$ whale. Furthermore, the degree that hydroxylated metabolites were detected was in the same order as the increasing in vitro biotransformation capacity. Jaspers et al. [110] found that aquatic and terrestrial predatory avian species differed in the HO-PCB profiles, with grey herons (*Ardea cinerea*) having relatively higher concentrations of HO-CB138 and common buzzards having higher concentrations of HO-CB146, with long-eared owls (*Asio otus*) and sparrowhawk (*Accipiter nisus*) having relatively higher concentrations of HO-CB187. Although they did not assess metabolic capability in these species, they argued that differences in dietary sources and species-specific metabolism of PCBs were the most likely causes of the observed HO-PCB profiles.

The major metabolites of PCDDs/Fs are hydroxylated, glucuronide and sulphate conjugates [111]. The majority of the hydroxylated PCB (OH-PCBs) or pesticide metabolites formed following Phase I (hydroxylation) or the conjugates formed during Phase II (conjugation) metabolism are excreted from the body [93].

Nonetheless, hydroxylated PCBs or PBDEs can displace native thyroid hormones by binding to the thyroid transporting protein transthyretin [112], which can exacerbate the elimination rates by reducing the rate that those few hydroxylated POPs are eliminated.

Although burdens of metabolites of POPs that have been found in animal tissues are often considered to be due to direct formation of metabolites from endogenous MFO action, in some cases the presence of metabolites were from dietary sources, rather than endogenous metabolism of the respective parental POP. Following an in vitro assay with polar bear hepatic microsomes to assess OH-PBDE formation, the estimates of oxidative metabolism of PBDEs was low enough to indicate that the OH-PBDEs in bear tissue was from dietary accumulation from seals as the main source of OH-PBDEs [113]. Although hydroxylation decreases the solubility for the conjugated molecule, for such lipophilic compounds the effect is small enough – a reduction in log K_{OW} of only approximately −1.4, as estimated using the fragment method for calculating log K_{OW} – and the metabolites are still essentially lipophilic and bioaccumulative, baring high rates of Phase II conjugation.

4.1.2 Metabolism of PCBs

The family of cytochrome P450 responsible for metabolizing PCBs depends on the chlorine substitution pattern for each congener. Metabolic rates also vary dramatically amongst PCB congeners, as Brown [114] estimated that "...individual PCB congeners exhibit a million-fold range in metabolic susceptibility... in humans...". PCB 153, for example, can be metabolized and has a number of different possible metabolites (including not only hydroxylated forms of 153 but also hydroxylated 146, due to NIH shift). However, the rates of metabolism for PCB 153 and other recalcitrant PCBs are nonetheless very slow, relative to the so-called "metabolizable" PCB congeners. PCB congeners are generally categorized as nonmetabolizable by cytochrome P450 enzymes if they lack both meta (*m*), para (*p*) and ortho (*o*),*m* vicinal H-atoms (Group I), metabolizable by cytochrome P450 2B enzymes if the congeners have *m*,*p* vicinal H-atoms (Group II), metabolizable by cytochrome P450 1A enzymes if they have *o*,*m* vicinal H-atoms (Group III) and metabolizable by both cytochrome P450 1A and 2B enzymes if they have both *m*,*p* and *o*,*m* (Group IV; [115]). Generally, higher chlorinated PCBs are more resistant to metabolism and hence have higher retention [92]. The ability to metabolize particular PCB congeners can vary substantially amongst species, even within the same class (e.g. mammals). Cytochrome P450 1A1 of humans, for example, show little ability for metabolizing PCB 126, yet rats, with a slightly different form of 1A, are capable of forming a number of hydroxylated PCB metabolites of PCB 126, such as 4-OH-3,3′,4′,5-tetra CB and 4-OH-3,3′,4′,5,5′-penta CB [116].

The relative ability of different mammals and avian species were examined by Imaeda et al. [117], using the ratios of the concentrations of HO-PCBs to parental PCBs, the higher the ratio, the greater the capacity to metabolize PCBs. For example, Baikal seals had a relatively low HO-PCB/PCB ratio of 0.047 and thus

were considered to have the lowest metabolic capacity amongst the species examined. The HO-PCB/PCB ratios were highest for terrestrial mammals, including the domestic dog (*Canis familiaris*; 29; [118]), polar bear (*Ursus maritimus*; 26; [119]) and raccoon dog (*Nyctereutes procyonoides*; 4.3; [118]) followed by two species of birds (Laysan albatross (*Phoebastria immutabilis* [~0.56]) and black-footed albatross (*Phoebastria nigripes* [~0.395] [120])), whereas the lowest were marine mammals (common bottlenose dolphin [*Tursiops truncatus*] and melon-headed whale (*Peponocephala electra*); < 0.05; [121, 122]). Such conclusions should be tempered, however, by discrepancies in the estimates of the ratios due to the different number of HO-PCBs and PCBs measured in each study and furthermore the putative discrepancies between the relative rates of formation of HO-PCBs for each species, with the corresponding rates of elimination of same.

4.1.3 Metabolism of PCDD/Fs

PCDD/Fs, dioxin-like PCBs such as coplanar PCBs, coplanar polybrominated biphenyls and PAHs typically induce isoforms of the cytochrome P450 1 family, by first binding with the free-floating aryl hydrocarbon (Ah) receptor in the cytoplasm, migrating to the nucleus in an aryl hydrocarbon nuclear transporter complex and finally forming an adduct thereby upregulating cytochrome P450 1A mRNA [94]. These compounds are in turn largely metabolized by isoforms from the cytochrome P450 1 family, particularly cytochrome P450 1A [123], although cytochrome P450 1, 2 and 3 families all metabolize at least some forms of dioxin-like compounds.

Using mammalian cytochrome P450 embedded in yeast, Inui et al. [21] estimated the binding ability of each isoform with PCDD/Fs and coplanar PCBs. Cytochrome P450 1A readily metabolizes some of the lower chlorinated PCDD/Fs such as mono-,di- and tri-CDD/Fs, particularly 2,7-di-CDD and 2,3,7-tri-CDD [21]. Conversely cytochrome P450 2 metabolizes lower chlorinated PCDD/Fs that were chlorinated on one of the phenyl rings, such as 1-mono-CDD, 2-mono-CDD and 2,3-di-CDD, but not PCDD/Fs that were chlorinated on both phenyl rings, such as 2,7-di-CDD and 2,3,7-tri-CDD. Cytochrome P450 3 did not affect any of the tested PCDD/Fs, and none of the cytochrome P450s from the 3 tested families exhibited any meaningful metabolism of 2,3,7,8-tetra-CDD (TCDD) [21]. The failure to metabolize TCDD is related to its resistance to metabolism rather than an inability of cytochrome P450 1A to bind with the substrate. These results indicate that CYP1A1 can bind 2,3,7,8-tetra-CDD in its substrate-binding pocket but shows no detectable activity towards it. A number of particularly recalcitrant and toxic PCDD/Fs, such as 2,3,7,8-tetra-CDD, 1,2,3,7,8-penta-CDD and 2,3,7,8-tetraCDF, do have high binding affinity to cytochrome P450 1A2, but the cytochrome P450 activities are inhibited by their presence [22, 124], thus reducing the rate of metabolism of the PCDD/Fs.

4.1.4 Metabolism of Organochlorine Pesticides

Many organochlorine pesticides induce cytochrome P450s from the 2B and 3A families [94]. DDT isomers, such as *p*,*p*′-DDT and *o*,*p*′-DDT, are readily metabolized by the cytochrome P450s 2B1, 3A1, 2B6 and 3A4 [125], but not cytochrome P450 1A [126]. Chanyshev et al. [127] found that cytochrome P450 1A was statistically increased in the ovaries (but not the liver) of exposed rats, but the level of induction of cytochrome P450 1A was relatively trivial (14 × control) compared to its induction by other compounds, such as benzo[a]pyrene (1,700 × control) or compared to the induction of cytochrome P450 2B by DDT (400 × control). Although DDT and their main metabolites DDE and DDD induced cytochrome P450 activity, DDE is selectively retained, due to its high lipid solubility and high resistance to further metabolism. Thus, DDT and its metabolite DDD are readily metabolized (and eliminated), whereas DDE is generally not further metabolized [126] in most animals, although there are some exceptions. Polar bears are suspected to be able to metabolize the otherwise recalcitrant *p*, *p*′-DDE, largely based upon its lower than expected bioaccumulation relative to other wildlife in the same ecosystem and more definitively by their formation of methylsulfonyl metabolites of DDE [113]. More typically, DDE tends to be amongst the more readily accumulated pesticides in avian species (e.g. [73]), and the BMFs of DDE are predicted to be amongst the higher values compared to other organochlorine pesticides and PCBs [128].

Like DDT, most organochlorine pesticides induce cytochrome P450 2B and 3A activity and are also metabolized by the same cytochrome P450 enzymes. Technical chlordane, a mixture of *cis*-chlordane, *trans*-chlordane and heptachlor, are chiral pesticides that are found in two forms of enantiomers (non-superimposable mirror images). Both *cis*-chlordane and *trans*-chlordane are hydroxylated by cytochrome P450 enzymes to 3-hydroxychlordane, which is subsequently dewatered to 1,2-dichlorochlordene [129], which is then modified further to oxychlordane by epoxidation [130]. Oxychlordane is one of the more common forms of chlordane found in wildlife tissues, due to its recalcitrant nature. Furthermore, the production of racemic forms differed between rats that were pretreated with cytochrome P450 2B inducers (e.g. phenobarbital) or cytochrome P450 3A (e.g. dexamethasone) [130]. Dieldrin, another type of cyclodiene pesticide, also induces cytochrome P450 2B [131] and cytochrome P450 1A and 2A [132]. Mirex induced cytochrome P450 1A, 2B, 2E and 3A [133].

4.2 Case Studies of Wildlife Exposure to POPs

4.2.1 Saglek Bay

In 1953, a US Air Force base operated in the Canadian Arctic, at Saglek Bay, Labrador, Canada, and was used primarily as a radar station. The base was

Table 2 Geometric mean PCB concentrations in sediments (ng/g dw) and biota (ng/g ww) from Saglek Bay, Newfoundland and Labrador, Canada, from 1997 to 1999 (Adapted from [11])

Matrix	Zone 1	Zone 2	Zone 3	Zone 4
Sediment	884	10	22	065
Clams	285	126	30	n/a
Sea urchins	5,190	30	21	33
Sculpin (liver)	23,900	536	188	45
Sculpin (whole body – liver)	783	287	45	11
Black guillemot (egg)	32,900	5,240	1,010	390
Black guillemot (nestling liver)	3,550	590	79	25

transferred to the Canadian Forces and closed in 1971. Subsequently, in 1989 the base was reopened as a North Warning System Long Range Radar facility. In 1996, it was discovered that the soil and sediment was contaminated with PCBs [134]. Although the initial contamination was likely the soil along the beach, due to erosion and other transport processes, there was significant contamination of the nearshore sediments. The PCBs were primarily from Aroclor 1260 [11], with maximum concentrations of 62,000 ng/g (dw), and declining with distance exponentially from the epicentre to a minimum of 0.24 ng/g, over a distance of ~25 km. During a risk assessment of the base, the area was designated into a number of zones consisting of concentric circles around the epicentre of PCB contamination, each of decreasing PCB exposure. Concentrations of PCBs were measured in sediment and biota from each zone. Although concentrations in biota decreased from the epicentre (zone 1) to the furthest area (zone 4), concentrations of PCBs were consistently elevated at the higher trophic levels, from the aquatic to the terrestrial (though fish-eating) food chain (Table 2). At the lower trophic levels in the aquatic environment, clams (unspecific species) were always the least contaminated, followed by sea urchins (unspecific species). Shorthorn sculpin (*Myoxocephalus scorpius*), which feed largely upon invertebrates and small fish from the demersal zone, had higher burdens than the clams and sea urchins. Black guillemot (*Cepphus grylle*) eggs or chicks, however, had higher concentrations than the invertebrates.

When livers of guillemots and sculpin are considered, depending on the distance from the PCB epicentre, the relative body burdens varied (Table 2; [11]). At the most contaminated site, the beach area, the sculpin had almost seven times the burden than the guillemot in their livers, but at the furthest zones, the relative concentrations were more similar (Table 2). Although generally guillemot feed within a few kilometres of the colony, occasionally they may forage as far as 55 km from the colony [135]. Thus, it is likely that at least some of the egg burden, and thus some of the nestling liver burden, was influenced by the guillemots foraging a distance away from the PCB contamination, reducing exposure and therefore both the egg and nestling liver burden. The transfer of PCBs from avian females' body burdens to their eggs ranges typically between 2% and 22% of the females'

body burdens (c.f. [136]), depending on the females' reproductive strategy (e.g. income vs. capital breeding).

4.2.2 Hudson River

As an example of a site that was highly contaminated with PCBs, the 200-mile stretch of the Hudson River (New York, USA) is one of the largest superfund sites, defined as part of a US Federal programme established to remediate uncontrolled or abandoned hazardous waste sites. The Hudson River has the distinction of being one of the most PCB-contaminated rivers in North America [137, 138]. It has been estimated that the General Electric Company released between 0.45 and 2.3 million kg of PCBs (including both non-permitted and permitted discharges), primarily Aroclor 1242, from two capacitor manufacturing plants (Fort Edward and Hudson Falls) on the Upper Hudson River between 1950 and 1976 [139].

Although the mass of PCBs being released into the river had declined precipitously, from the initial 2,200–16,000 kg/year before 1977, in 1998 PCBs were estimated to be released at a rate of about 30 kg/year [10]. After earlier events such as the removal of a dam in 1973 and a large flood in 1976, following the collapse of a retention wall at the GE plant in Hudson Falls in 1991 resulted in the deposition of PCBs in sediment at the Thompson Island Pool, 10 km downstream of Fort Edward. In 1991, the highest PCB concentrations in surficial sediments were 2,000 μg/g (ww) at the Thompson Island Pool and 4,000 μg/g (ww) 8 km downstream of the pool near the Northumberland Dam [140].

Earlier reports indicated a very high level of contamination in wildlife from the Hudson River. The mean concentrations of PCB adult male snapping turtle adipose collected in the Hudson River between 1976 and 1978 was 2,990 μg/g (lw) [141]. Adipose from a single snapping turtle from the Upper Hudson River had 3,560 μg/g total PCBs [142]. More recent reports (2003–2005) still show elevated PCB concentration in snapping turtles from the Hudson River. Mean concentrations of PCBs ranged from 2.8 μg/g (ww or 38.9 μg/g lw) in snapping turtle eggs from the Thompson Island area to 0.059 μg/g (ww or 0.82 μg/g lw) in eggs from lakes and ponds north and west from the contaminated area [10]. The highest concentration was 12.1 μg/g ww (168 μg/g lw).

In 2004, PCBs, pesticides and PCDD/Fs were measured in the eggs of spotted sandpipers (*Actitis macularia*) collected from the Hudson River downstream of Fort Edward and on two rivers that flow into the Hudson River [143]. The (geometric) mean concentrations of PCBs (9.1 μg/g ww) in eggs from the Thompson Island Pool area were significantly higher than those of the other two rivers (0.6 μg/g ww each), with the maximum concentration found of 72.3 μg/g ww. Neither dioxins nor pesticides varied much amongst sites, with mean total PCDD/Fs, DDE and dieldrin concentrations varying between 0.009 and 0.006, 50.4 and 57.8 and 2.14 and 4.45 ng/g (ww), respectively [143]. Although PCDD/Fs were not elevated due to

the release of PCBs from the GE plants, dioxin-like activity was higher in the contaminated area. Using tree swallows as monitors at three sites in the Hudson River and far downstream of the Hudson Falls and Fort Edward plants, Secord et al. [138] found that the mean TEQs in the eggs near the affected sites had means ranging between 7.5 and 12.7 pg/g (ww), whereas the far downstream site had 1.7 pg/g and the control site had 3.1 pg/g. Nestlings provided a stronger discrimination amongst the sites than did the eggs. Mean TEQs of whole-body nestlings near the affected sites were 14.6 and 25.4 pg/g (ww) vs. the far downstream site of 1.4 pg/g and control site of 0.4 pg/g [138]. Hence, the mean TEQs of the whole-body nestlings from the area most affected by PCBs were 4.1× higher than that from the upstream control site but was 62× higher than that of the control site. The greater discrimination in PCBs and TEQs amongst sites by the nestlings compared to the eggs was likely due to some residual influence of the female's body burden. Given the long half-lives of typical POPs, including PCBs, at least some of the females' body burden would be from the females overwintering feeding. Typically, the plasma of chicks (or other measures of their body burden) better reflects influence from the local environment than do eggs due to their maternal influence [144].

4.2.3 Lake Apopka

A large spill of Kelthane, which was used primarily as a miticide or acaricide, was accidentally released into Lake Apopka in 1990. Although dicofol was the putative active ingredient of Kelthane, technical DDT was used as an intermediate in its manufacture; early manufacture of Kelthane contained up to 15% DDT or DDT metabolites [145], although later technical grade contained less than 0.1% DDT or metabolites. Following the accidental spill into Lake Apopka, the hatching success of alligators from Lake Apopka was reduced to approximately less than 20% from 1983 to 1991, compared to a more typical rate of hatching success of between 88% at a nearby control wildlife refuge area in the same time period [146]. In 2001, about 10 years after the spill, livers from juvenile alligators were sampled from Lake Apopka and Lake Woodruff National Wildlife Refuge. Concentrations of *p*, *p*′-DDE ranged between 4.3 and 778 ng/g (ww), whereas the livers from the control contained only 0.6–4.6 ng/g [146]. Conversely, although the majority of the compound spilled was dicofol, only small amounts of dichlorobenzophenone (DCBP) were detected in the alligator liver. Techniques at the time were unable to resolve dicofol [146] as it thermally degrades to DCBP [147] as well as be metabolically transformed into DCBP [148], at least in mammals. From 1983 to 1986, egg viability of alligators from Lake Apopka declined, whereas in other lakes surveyed there was no trend [149]. The density of juvenile alligators from Lake Apopka fell during the same time period.

5 Temporal Trends of POPs in Wildlife

Given that the production of legacy organochlorine pesticides has stopped in most countries, manufacturing of PCBs has ended worldwide, and there have been controls on the inadvertent production of polychlorinated dibenzo-*p*-dioxins, and furans, in general concentrations, have declined not only in abiotic matrices but in the body burdens of wildlife as well.

Note, however, that declines have not always been apparent, and despite reductions in emissions, or at least putative reductions, body burdens of POPs in wildlife have not always responded.

5.1 PCDD/F Emissions and Cormorant Exposure

In 1999, a report was published that indicated that out of 15 nations monitored, PCDD/F emissions in air in 1995 was the highest in Japan, reaching almost 4 kg TEQ/year, which was almost twice as high as the USA, the country with the second highest emissions [150]. A study by Kubota et al. [151] was designed to determine the success of a 1999 national regulation that was implemented in Japan to reduce dioxin emissions, by examining the concentrations of PCDD/Fs and non-*ortho* PCBs in the livers of common cormorants (*Phalacrocorax carbo*). Simultaneously, to complement the chemical analyses of selected dioxins and furans, they also monitored biomarkers of dioxin-like activity in the birds, by using a measure of cytochrome P450 1A response. Although there was an initial drop in emissions of dioxin-like compounds, as estimated by TEQs, from approximately 2 kg TEQ/year in 2001 to about 0.45 kg TEQ/year in 2003, estimated emissions declined only very slowly from 2001 to 2008 [152]. The total TEQs in the cormorant livers varied between 8.2 and 1.2 ng/g (lw) in males and 7.2 and 1.3 ng/g (lw) in females [151], and the majority of TEQs was due to the presence of non-*ortho* PCBs. Except for the values being highest for both females and males in 2001, there was no clear reduction of PCDD/Fs or cytochrome P450 1A activity in cormorant livers. They concluded that body burdens of PCDD/Fs in cormorants did not respond significantly to the national regulation on dioxin emissions.

In general, however, due to the reduction in the production and emissions of legacy POPs, both exposure and hence body burdens in wildlife have generally decreased.

5.2 Spatial and Temporal Trends of POPs in Polar Bears

Polar bears have been monitored in the Arctic for legacy contaminants since the 1980s. Polar bears are large carnivores that live in low densities throughout the

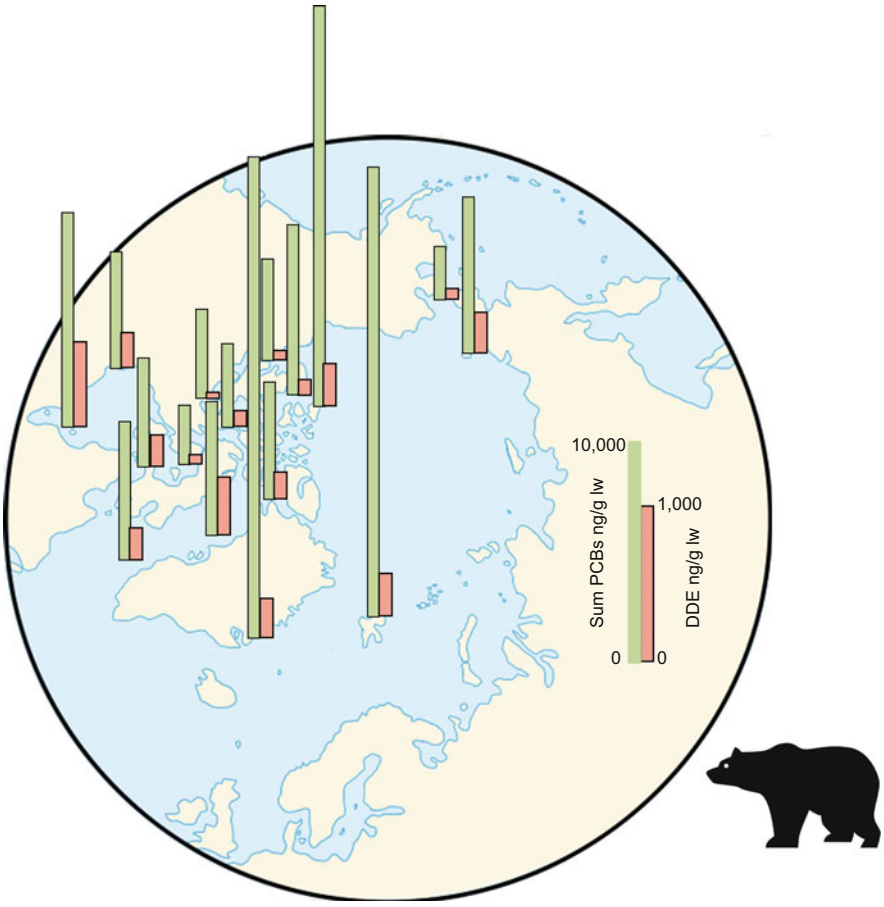


Fig. 3 Mean concentrations of polychlorinated biphenyls (PCBs) and 1,1′-(2,2-dichloro-1,1-ethenediyl)bis(4-chlorobenzene) (*p*,*p*′-DDE) (ng/g wet weight) in polar bear (*Ursus maritimus*) adipose in the Arctic, 1988–1993. Figures based upon [153]

polar basin, including the USA, Canada, Norway, Finland and Russia. As top predators, generally feeding on mammalian predators of fish (seals), they have amongst the highest potential of dietary exposure to POPs [153].

PCBs are the dominant legacy compounds in the adipose of polar bears (Fig. 3). Concentrations of PCBs were highest, in general, in populations near the McClure Strait and the adjacent Arctic Ocean, eastern Greenland and Svalbard. DDE concentrations were highest in the lower Hudson Bay and on the west side of Greenland; however, although there were differences in pesticide concentrations in polar bear adipose amongst regions, the differences were relatively small compared to those observed with PCBs. The highest concentrations of mean PCB concentrations in bear adipose in the 1990s was off the east coast of Greenland near Scoresbysund, at 24.3 μg/g (lw), whereas the highest mean concentration of DDE was 0.513 μg/g

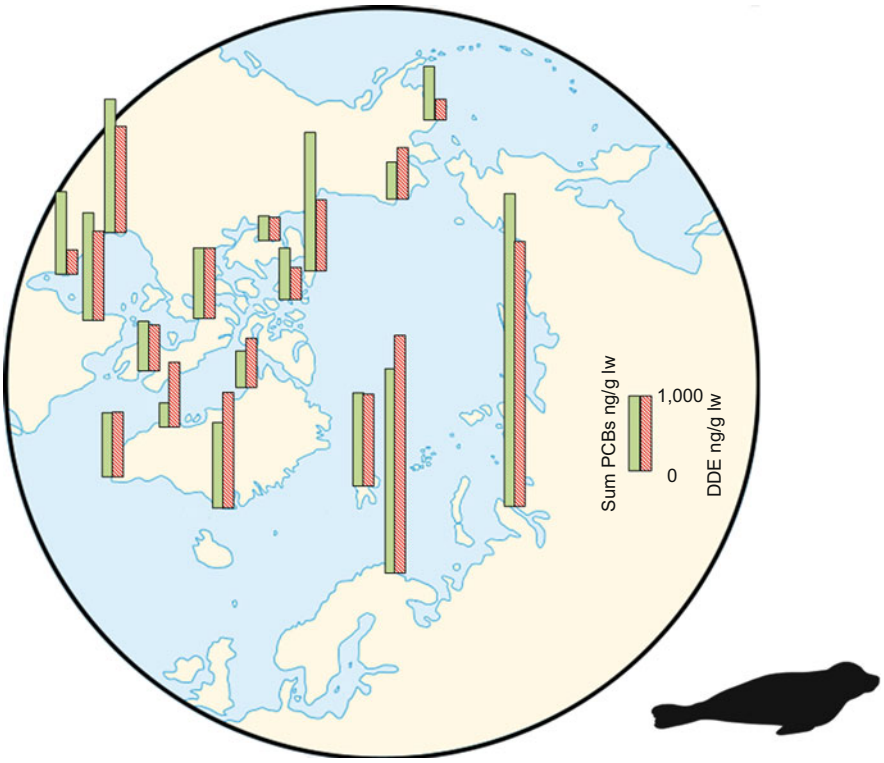


Fig. 4 Mean concentrations of polychlorinated biphenyls (PCBs) and 1,1′-(2,2-dichloro-1,1-ethenediyl)bis(4-chlorobenzene) (*p*,*p*′-DDE) (ng/g wet weight) in ringed seals (*Phoca hispida*) adipose in the Arctic, 1988–1993. Figures based upon [153]

(lw), off the eastern Hudson Bay by the Belcher Islands. Among some of the same general sites, concentrations of TCDD varied between < 2 and 23 pg/g (lw), in 1983–1984.

A different pattern emerges when considering their main prey, the ringed seal. Concentrations were very similar between DDE and PCBs within sites (Fig. 4), likely due to the relatively poorer ability of the seals to metabolize and thus eliminate *p*,*p*′-DDE, at least compared to polar bears. PCB concentrations were highest in seal blubber from Yenisey Gulf, in Arctic Russia, with a mean of 4.2 μg/g (lw), followed by those from Jarfjord off the coast of Norway; these two sites also had the highest DDE concentrations in seals. BMFs for polar bears from seals, calculated from two regions (Svalbard and Lancaster Sound), ranged between 3.7 and 10 for chlordane and 7.2 and14 for PCBs but were only 0.3 for sum DDT [153]. Conversely, BMFs for seals from cod for chlordane, PCBs and DDT ranged between 3.9 and 5.8, 7 and 48 and 3.9 and 36, respectively. Gulls, however, had BMFs between 172 and 208 for DDT [153].

From 1983 to 2010, 19 legacy contaminants have been monitored in adipose of polar bears from East Greenland [154]. Sum PCB concentrations in adipose of subadult bears declined from 22.7 μg/g lw to 8.47 μg/g lw during this period, and α-HCH had the greatest rate of decline, falling an average of 10.8% a year over a 27-year period. These rates of decline were similar to those found elsewhere, albeit over shorter periods of time. Norstrom et al. [155] and Verreault et al. [156] reported 32–75% declines for 1989–1993 to 1996–2002, respectively. *p*,*p*′-DDE concentrations have also been reported to have declined from 1989 to 2008 in most polar bear subpopulations [157]. However, despite early declines, from 2000 to 2010 there was no further apparent decline in body burdens [154].

The diet of polar bears has, unsurprisingly, also shown declines in body burdens. Following an exponential decline (DDT starting in 1981, PCBs starting in 1970), the half-lives of *p*,*p*′-DDT and *p*,*p*′-DDE in female ringed seals from Arctic Canada were 9 and 36 years, respectively, while those of PCB half-lives were 20–60 years [158]. Similarly, Wolkers et al. [159] determined that POPs such as PCBs and organochlorine pesticides in ringed seals from Svalbard, Norway, were between 50% and 90% lower in 2004 compared to those from 1996. The decline in ringed seals has been observed in other Arctic seals. For example, Ross et al. [160] found that for PCBs and polychlorinated naphthalenes, concentrations in the blubber of harbour seal (*Phoca vitulina*) pups have been declining in an exponential fashion from the Salish Sea, North America, from 1984 to 2003.

5.3 Aerial Insectivores

Using a rather unconventional monitoring taxonomic group – bats – Bayat et al. [161] examined changes in pesticide burdens in bat tissues worldwide, mostly in the USA but also in Australia, India and a few European countries. Most bats are aerial insectivores, catching small flying insects, although a number are frugivores. Given the differences in methods, tissue sampled and particularly species (with all the differences due to metabolism and diet that entails), the trends reported have to be taken cautiously. Nonetheless, the body burdens of pesticides in bats have declined; the minimum and maximum ranges in DDE concentrations were 2.6–62, 0.05–2.31 and 0.08–0.19 (μg/g ww), during the time periods 1970–1980, 1981–1999 and 2000–2013, respectively. Dieldrin, heptachlor epoxide and some components of chlordane also declined, though not generally as much as DDT and its metabolites. Custer et al. [162] examined the trends of POPs in tree swallow (*Tachycineta bicolor*), an avian aerial insectivore, at the Sheboygan River, Wisconsin (USA), both upstream and downstream of a source of PCBs. From 1990 to 2010, sum PCBs declined from 8.69 down to 3.27 (μg/g ww) at the downstream site.

5.4 Colonial Waterbirds

In the early 1970s, many bird populations, including colonial waterbirds in the Great Lakes, had high incidences of deformities and had reduced productivity; these adverse reproductive effects were found to be prevalent in herring gulls and other fish-eating colonial waterbirds nesting in Lake Ontario and other areas of the Canadian Great Lakes [163]. Due to these problems, Environment Canada began using herring gulls, as indicators of health and contaminant exposure for fish-eating wildlife in the Great Lakes [164]. The Great Lakes Herring Gull Egg Monitoring Program (GLHGEMP) remains one of the longest-running contaminant programmes in the world. Other species, however, such as double-crested cormorants (*Phalacrocorax auritus*), black-legged kittiwakes (*Rissa tridactyla*) and thick-billed murres (*Uria lomvia*), have also been monitored for contaminants across Canada.

When comparing the concentrations of PCBs in fish-eating birds across Canada, including the Arctic, using herring gulls, cormorants, great blue herons (*Ardea herodias*), thick-billed murres, northern fulmar (*Fulmarus glacialis*), black-legged kittiwakes and Atlantic puffins (*Fratercula arctica*), it is clear that in general the Great Lakes birds have not only the highest concentrations but also the greatest variance (with the mean sum PCBs per colony ranging between 1.8 and 15 μg/g ww; Fig. 5), followed by the St. Lawrence River (which is immediately downstream of the Great Lakes), with mean PCBs in eggs from six colonies of herons ranging from 0.96 to 6.1 μg/g ww per colony [168]. The Great Lakes region is a very large watershed, which contains some of the most intense agricultural and industrial densities that contribute to the degree of POP contamination. Colonial waterbirds from the Arctic had much lower egg burdens of PCBs compared to those from across Canada, with mean concentrations in the eggs of thick-billed murres ranging between 0.017 and 0.061 μg/g (ww) per colony and between 0.09 and 0.35 μg/g (ww) for northern fulmars in 2007 [165]. Similar patterns exist for organochlorine pesticides, in general, in eggs amongst these colonial waterbirds from the Atlantic to the Arctic and to the Pacific.

Of the nine species monitored at the Yellow River, China, Saunders's gulls (*Larus saundersi*) had the highest concentrations of PCBs and pesticides [169]. The Yellow River Delta is a biosphere reserve of the China Biosphere Reserve Network and represents an important bird breeding and overwintering area. However, this region is also an important chemical and petrochemical industry base, with substantial sewage and waste discharges entering the river. Assuming that the gull eggs had the same % lipids as herring gulls (~6.5%), sum PCBs averaged 0.72 μg/g (ww), whereas sum DDT averaged 0.04 μg/g (ww). Morales et al. [170], using pooled egg samples from yellow-legged gull (*Larus michahellis*) and Audouin's gull (*Larus audouinii*) from three colonies each at the Punta de la Banya, Ebro Delta Natural Park, Spain, in 2010, measured PCBs, organochlorine pesticides and non-legacy POPs such as flame retardants. They found that sum PCBs (of only 7 congeners) and *p*,*p*′-DDE varied from 0.718 to 0.790 μg/g (ww) and 0.019 to

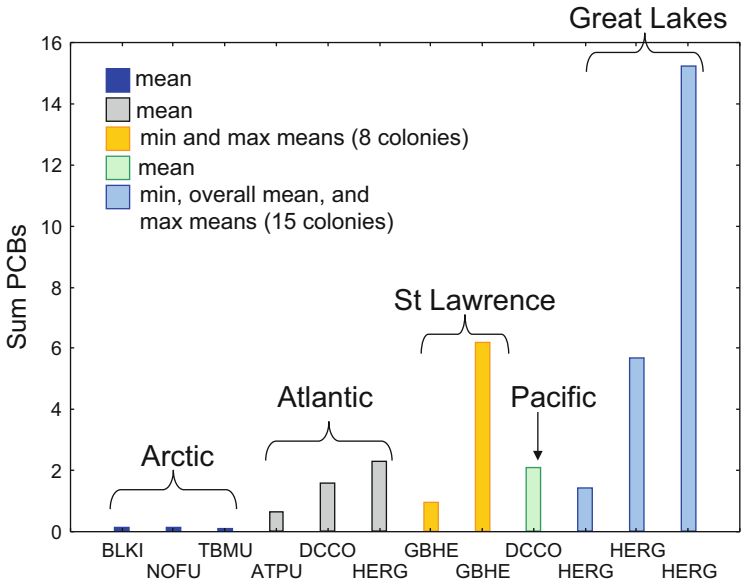


Fig. 5 Mean concentrations (μg/g, ww) in the eggs of colonial waterbirds in Canada and the USA, divided within regions (Arctic, Atlantic, St. Lawrence River, Pacific and Laurentian Great Lakes). Species include *HERG* herring gull, *DCCO* double-crested cormorant, *GBHE* great blue heron, *ATPU* Atlantic puffin, *TBMU* thick-billed murres, *NOFU* northern fulmar and *BKLI* black-legged kittiwake. Data from [165–167]

0.027 μg/g (ww), respectively, in eggs from yellow-legged gulls. The egg burdens were similar in Audouin's gull eggs from the same sites, with sum PCBs and *p*, *p*′-DDE varying between 0.348 and 0.680 μg/g (ww) and 0.016 and 0.021 μg/g (ww), respectively.

Although those concentrations were much lower than those found in the Laurentian Great Lakes area, historically, concentrations were much higher than those found by Morales et al. [170] in 2010. Audouin's gull eggs collected in 1988 had an average of 3.46 and 9.05 μg/g (ww) PCBs, at Chafarinas Islands and the Ebro Delta, respectively, and 2.48 and 2.64 μg/g (ww) *p*,*p*′-DDE, respectively [171], indicating that there was a large decline over the 22 year interval. Fliedner et al. [172] examined the temporal trends of PCBs in herring gulls at three colonies in coastal Germany, from 1988 to 2008. At the most contaminated colony, located on Trischen Island, in the North Sea, concentrations fell from 1.76 μg/g (ww) in 1988 to 0.38 μg/g (ww) in 2008. Similarly, sum PCBs in eggs from Mellum fell from 1.01 to 0.23 μg/g (ww) during the same time period, while those from Neuwiese fell from 0.9 (in 1996) to 0.46 μg/g (ww; [172]).

Similarly, in a 40-year study on POPs in herring gull in the Great Lakes, de Solla et al. [166] showed that PCB concentrations in eggs dropped significantly in each of 15 colonies. Fighting Island (Detroit River, ON) had the highest concentrations of PCBs, in 1972, of 52.47 μg/g (ww), which declined to 9.80 μg/g (ww) in 2013. The

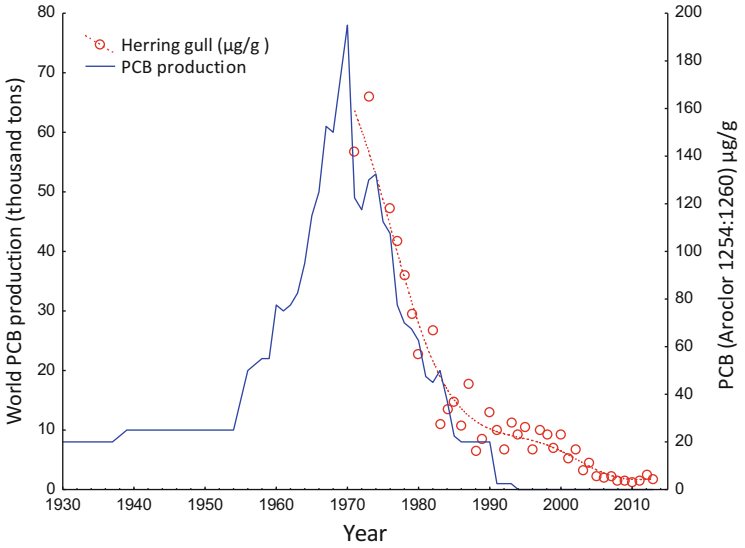


Fig. 6 Overlay of the world's annual production (in thousand tons) of polychlorinated biphenyls (PCBs) (1930–1992) and concentrations of PCBs (as Aroclor 1254:1260 (1:1) equivalents, ug/g, wet weight) in herring gull (*Larus argentatus*) eggs from the Canadian Laurentian Great Lakes. PCB production from Breivik et al. [3]) and herring gull data from de Solla et al. [166]

least-contaminated colony for PCBs in 1974, Chantry Island, had concentrations in gull eggs decline from 3.97 to 1.49 µg/g (ww) in 2013. The site that had the highest TCDD egg burdens in 1981, Snake Island (Lake Ontario, Canada), declined from 185 to 9.7 pg/g (ww) in 2013. For pesticides, the percent declines ranged from 72.7% for sum chlordane to 95.2% for mirex. Interestingly, the trajectory of PCB burdens (as expressed as Aroclor 1254:1260 1:1), in herring gull eggs from the Great Lakes, mirrored the estimated worldwide annual PCB production, given a short lag time (Fig. 6), although it should be noted that most PCBs were manufactured in the USA (Table 1). Braune et al. [173, 174] also documented declines of POPs in colonial waterbird eggs in the Arctic, from 1975 to 2003 in colonial waterbirds. Of the three species monitored, black-legged kittiwakes had the highest contaminant burdens. Sum PCBs, DDE and chlordane declined from 1.66, 0.243 and 0.58 µg/g (ww) in 1975 to 0.177, 0.044 and 0.045 µg/g (ww) in 2003.

5.5 Obligate Piscivores

Osprey (*Pandion haliaetus*) are obligatory piscivorous raptors, whose populations are found on every continent, Antarctica excepted, and they feed on fish from either marine or freshwater bodies of water. Like many other piscivorous birds, their

populations throughout North America, Europe and elsewhere had dramatic population declines, largely due to eggshell thinning caused by DDT. Due to its sensitivity to contaminants and its widespread range, many governmental organizations such as the US Geological Survey and US Fish and Wildlife Service (USA), Environment Canada (Canada) and Brandenburg State Agency (Germany) and scientists from other organizations have often used osprey as environmental monitors for tracking POP burdens and their effects. Contaminants in osprey have been monitored in Canada, in the USA, and throughout Europe. Even though some data are available from other time periods [74], I compiled a subset of the data on concentrations of PCDs, PCDD/Fs and pesticides in osprey eggs from the 1990s onwards to maximize the comparability amongst sites.

Concentrations of PCBs in osprey eggs varied by about 2 orders of magnitude worldwide, but the highest concentrations were found in an area of low industrial or manufacturing density. In a study examining PCBs, PCDD/Fs and pesticides in osprey eggs in the Canadian Great Lakes area in the early 1990s, one of the sites (Sturgeon Lake, ON) had what appeared to be an Aroclor 1254 point source at the side of a conservation area [9], which was reflected in the eggs and plasma of osprey chicks. Mean concentrations throughout the rest of the study areas ranged between 1.65 and 4.53 μg/g (ww) but averaged 7.09 μg/g at Sturgeon Lake (Table 3). Given the point source nature of the PCB source, the variance in contamination was very high, and concentrations in individual eggs varied from 2.02 to 26.54 μg/g ww [181]. Despite the Aroclor source, PCDD/Fs did not appear to be elevated at the contaminated site. Non- and mono-*ortho* PCBs were also similar amongst the different colonies, except for PCB 77 and 81, which averaged 120 and 479 pg/g (ww), respectively, throughout the Great Lakes but averaged 771.5 and 1,088 pg/g (ww), respectively, at Sturgeon Lake [181]. The source of Aroclor 1254 in Sturgeon Lake was never discovered.

PCB contamination in osprey eggs was similar throughout much of the Great Lakes [181], in Western Canada and the USA at Columbia River [183]. Conversely, concentrations tended to be a bit lower in Europe and much lower in Australia, with mean PCB concentrations at two separate colonies of 0.223 and 0.023 μg/g (ww; Table 3). Conversely, TCDD was highest in Wisconsin (mean 0.108 ng/g ww), where the osprey colonies were downstream from two bleached-kraft mill facilities, which are known sources of PCDD/Fs. Although TCDD was not elevated, the sum of PCDDs was highest in eggs at Upper Willamette River, primarily due to OCDD (mean 1.3 ng/g ww); although the source of this dioxin was unknown, pentachlorophenol had been used locally upstream as a wood preservative and could be a potential source.

In what has been the longest-running osprey contaminant monitoring study, sum DDT and sum PCBs in osprey eggs from Norway declined from 68.7 and 7.82 μg/g (ww) in 1970–1974 to 0.40 and 0.66 μg/g, respectively, in 2000–2005 [176]. Although it appeared that other pesticides have declined as well, those changes were not as dramatic, largely due to the lower concentrations overall but also since those others were not measured until 1990 onwards.

Table 3 Concentrations of polychlorinated biphenyls, organochlorine pesticides and polychlorinated dibenzo-*p*-dioxins and furans in osprey (*Pandion haliaetus*) eggs throughout the world. Units are in ug/g wet weight with the exception of PCDF, PCDD and 2,3,7,8-TCDD which are in ng/g

Country	Site	Year	Sum PCBs	DDE (or sum DDT)	Chlordane	Dieldrin	Mirex	HCB	PCDF	PCDD	2,3,7,8-TCDD
Russia [175]	Volga River	1992	0.111	0.25							
Norway [176]	Unknown	1990–1994	1.787	0.855	0.003	0.0048	0.0002	0.0012			
Spain [177]	Menorca	1994–2000	0.594	0.542					0.0039	0.0035	0.0003
Germany [178]	Brandenburg/ Mecklenburg	1992–1995	0.296	0.174							
USA [179]	Delaware Bay	2002	4.186	1.078	0.0005	0.0018	0.0006	>0.0005			
USA [73]	Upper Willamette River, OR	1993	0.688	2.347	0.00167	0.0004	0.0003	0.00038	0.009	0.170	0.0023
USA [180]	Wisconsin River, WI	1993	1.666	0.526							0.108
Canada [181]	Sturgeon Lake, ON	1991–1992	7.089	1.361	0.00597	0.00164	0.0021	0.00016	0.042	0.026	0.0027
Canada [181]	Georgian Bay, ON	1991–1992	3.702	3.265	0.0107	0.00233	0.0054	0.00037	0.112	0.088	0.0055
Canada [182, 183]	Nechako River, BC	1992	0.104	0.138	0.00035	0.00035	0.00008	0.00011	0.0014	0.222	0.0013
Canada [182, 183]	Columbia River, BC	1993	0.175	0.377	0.0034	0.00025	0.00049	0.00024	0.0273	0.0638	0.021
Australia [184]	North Coast	1987–1988		0.223		0.00267					
Australia [184]	Rosemary Island	1988		0.023							

6 Wildlife as Monitors of Environmental Contamination

If the only question being asked was how much of a contaminant is in the environment, the simplest and most effective way to determine the answer is to measure the contaminants in whatever environmental matrix was of interest, be it soil, sediment, water or air. Regardless of the environmental component that any particular contaminant has an affinity for (again, soil, sediment, water or air), any mass-balance analyses would reveal that the mass found in wildlife tissue as compared to the appropriate environmental matrix is very small. Why then monitor wildlife then for POPs, aside from assessing toxicity? The bioavailability of POPs and thus exposure to wildlife are not simply a function of the concentrations found in environmental matrices but vary considerably with the myriad of factors that control the transport and fate of contaminants. Extreme weather events can dramatically increase the bioavailability of POPS, such as the influence of typhoon events on PCDD/F remobilization into the atmosphere, thus increasing long-distance transport [185]. Differences in organic carbon content in soil or sediment can have dramatic influence on the availability of POPs contained therein to biota. Dredging events frequently increase the bioavailability of POPs, even if the mass of the contaminants are reduced due to remediation [186–188]. Models examining global climate change predict changes in emissions, fate and transport of POPs in at least some regions, particularly in the Polar Regions where local availability of some POPs is expected to increase with further warming [189, 190]. Changes in food webs affect exposure and thus body burdens in wildlife, to a degree such that monitoring programmes may over- (or under-) estimate long-term temporal declines in PCBs and organochlorine pesticides in fish-eating birds [66, 191]. Measurements of body burdens in wildlife integrate the net effect of factors such as bioavailability, temperature, growth rates, food chain dynamics and chemical partitioning behaviour, amongst others.

6.1 Response Rates of Wildlife vs. Abiotic Matrices to Flux of POPs

Why monitor wildlife, rather than water, soil or sediment for POPs? Other than confirming actual exposure to wildlife, which depends not only on the amount of POPs in the environment but also availability of the POPs to wildlife, which is not easily estimated, POP burdens in wildlife track changes faster than abiotic matrices. The rates of elimination of body burdens for POPs are generally much faster than the rates of environmental degradation; hence, changes in body burdens reflect changes in the bioavailability of POPs. For example, degradation half-lives of tri- to hepta-chlorinated PCBs in sediment were estimated to range between 3 and 38 years [192], with the congeners frequently found in wildlife tissues having half-lives of 10–19 years in sediment. PCBs are naturally degraded slowly over

time by microbial processes and profiles can change as certain PCB congeners are subject to reductive dechlorination which has been shown to occur primarily at the meta- and para-positions [193, 194].

Doick et al. [195] estimated that the half-lives of PCBs 28 and 52, two relatively readily degraded congeners, in soil were 10.9 and 11.2 years, respectively. Similarly, the half-lives of PCB congeners 18 and 28 were <1–8.5 years, in a 25 years study of sewage sludge-amended agricultural soils [196]. An earlier study did find shorter half-lives for tri- and tetra-CBs of 0.75 and 3.2 years, respectively, from sludge-amended farmland over 5 years [197]. As these studies focused on amended farmland soils where they were treated with sewage sludge, it is likely that these values would differ substantially from natural, undisturbed soils. Although Ayris and Harrad [198] found a very wide range of the mean half-lives of PCBs in soils (0.24–23.7 years), depending on congener (28, 52, 101, 138 and 180), soil amendment and soil type, they found that the weathered soil that they "consider the most applicable to 'typical' UK conditions" had an average half-life of 5.5 years. At a site where DDT had been applied to as late as 1970, the half-lives for the degradation of DDT into DDE were estimated to be between 20 and 50 years [199]. Sinkkonen and Paasivirta [192] used models to calculate predicted half-lives of PCBs and PCDD/Fs in the Baltic Sea area with an average temperature of 7°C, given both photolysis and biodegradation in soil and sediments but with no other losses and estimated that the half-lives of PCBs would range from 3 to 38 years and for PCDD/Fs would range from 51 to 273 years. Presumably realistic values would be lower, given more typical weathering and a wider range (and higher maximum) temperature of natural soils and sediments, as well as considering losses to other environmental compartments.

Conversely, the half-life of *p*,*p*′-DDE in herring gulls tissues was estimated to be 264 days [200, 201], with half-lives for PCBs likely to be similar. Similarly, the half-lives of PCBs fed to ring doves ranged from 7 to 53 days [202]. Bioaccumulation through diet tends to be rapid. POPs in the Hamilton Harbour area have been shown to be bioavailable to wildlife feeding in the area. Adult and juvenile farm-raised mallards (*Anas platyrhynchos*) were raised and then released in the Hamilton Harbour Confined Disposal Facility (Hamilton Harbour, Ontario, Canada), which contained high levels of PCBs and other contaminants [203]. The birds were enclosed and allowed to feed on the food that was locally available; after only 10 days, the birds had PCB concentrations in breast muscle that were 5,300 times greater than on the first day of exposure [203]. Conversely, the half-lives as estimated in declines in the eggs of free-ranging birds (years) is much longer than the half-lives as estimated by depuration of dosed birds (days to months). Based upon 40 years of monitoring POPs in herring gulls from 15 colonies through the Laurentian Great Lakes (North America), the average half-life of sum PCBs was 13.4 years, whereas they ranged between 5.46 (HCB) to 13.67 (chlordane) for pesticides and 7.9 years for TCDD [166]. Since the depuration rates of POPs in birds are relatively short compared to the rate of change in POP environmental stores, the rate of annual change in body burdens of birds are likely determined by environmental exposure, not internal metabolic processes.

6.2 Role of Tissue Type on Interpretation of Body Burdens

The most frequently used tissues for monitoring contaminant burdens in wildlife are egg, the liver, adipose or plasma; other tissues are sometimes used, such as the muscle, whole body, kidney, brain and feathers. Due to differences in lipid content, protein availability and cellular turnover, measuring contaminants in different tissue types can alter the nuances in the interpretation of body burdens, particularly for long-lived and migratory species. The equilibrium lipid-partitioning theory predicts that the concentrations of lipophilic compounds should be identical amongst tissues when expressed on a lipid basis [28]. Although the ratios of the concentrations of lipophilic contaminants were approximately 1 amongst tissue types in fish [28], the ratio of concentrations between eggs and maternal muscle of oviparous species, such as reptiles and birds, deviated from the predicted ratio of 1. Other studies have also found that the equilibrium lipid-partitioning theory does not hold for other mammals and birds [9, 119, 202, 204].

Furthermore, the reproductive strategy whereby the female allocates energy resources into reproduction may affect POP accumulation, by conflating POP accumulation from feeding at the wintering grounds with exposure from the breeding grounds. At the risk of oversimplifying matters, reproductive allotment strategies amongst species can be classified into two main groups: income and capital breeders. Income breeders do not deplete maternal lipid reserves during egg formation, but instead energy acquired while feeding at the breeding grounds is used as the primary energy source for egg development [205]. Capital breeders, conversely, use already-existing lipid stores that were acquired prior to the breeding season as an energy source for egg development [205]. Capital breeding in ectotherms, including fish, reptiles and amphibians, is relatively common [206], whereas income breeding is relatively common in avian species. Local contaminant sources can be masked by maternal burdens in eggs, particularly in migratory species (e.g. many birds, seas turtles, some mammals and fish), and to some extent, for capital breeders. Hence, the maternal burden source of contaminants that is deposited in the eggs may reflect both the recent female diet that was acquired during the breeding season and the reallocation of female lipid stores that represents earlier contaminant exposure and acquired body burden. Avian species that invest between 5% and 18% of their maternal lipid reserves into the development of the eggs generally have an egg/maternal ratio of PCB concentrations between 0.3 and 0.7 [202]. Conversely, female birds that do not deplete their lipid reserves during the period of lipid deposition into the developing eggs form the egg lipids from those acquired from their diet [207].

The initial body burdens of nestlings are from the burdens in the eggs (obtained solely from the burden of the laying females), but the subsequent feeding of the chick ultimately contributes more to the burden of the chick than the initial maternal burden. Furthermore, the initial egg burden is diluted by the growth of the chick, and the birds do metabolize and eliminate some of their initial burdens. The dietary intake of contaminants by the nestlings may have a greater effect on the

nestling's body burden than does the effect of the growth dilution [144]. Plasma of chicks is more representative of the contamination in the local area, due to the feeding of the chicks from the surrounding foraging area, than do eggs [144], and this relationship holds even when the nestlings and eggs are from the same nest [9]. Similarly, using a bioenergetics-based model, Nichols et al. [208] found that PCB burdens increased with the age of nestling tree swallows, a small insectivorous passerine, from contaminated sites on the Upper Hudson River, New York, and concentrations of 15-day-old chicks were about four times higher than the burdens in eggs. Generally, the model fit well when PCB concentrations measured in food boli from the adults were included in the model. In a few cases, however, opposite trends between body burdens and initial egg burdens were observed. Dauwe et al. [209] demonstrated that the concentrations of PCBs and the most prevalent organochlorine pesticides in eggs of great tits (*Parus major*), another small insectivorous passerine bird, were 4–6 times higher than those of whole-body nestlings. Hence, they found that the majority of POP burdens in near fledgling nestlings were from the initial egg burden, as opposed to coming from a dietary source of the nestlings. It is possible that some of the egg burden was from maternal lipid reserves when the females were feeding from a more contaminated area than the breeding grounds, which would confound the results.

Although in many cases it is logistical constraints that dictate the sampling regime and the selection of tissues targeted for sampling, one should select the most appropriate matrix for the answering the question at hand. If the objective is to characterize the influence of contamination in the local environment, tissues that best reflect the contamination from the local foraging area, such as plasma or newly grown feathers, are best used for monitoring. As stated earlier, in general, predictions from the equilibrium lipid-partitioning theory indicate that the concentrations of POPs should be sequestered equally amongst tissues when expressed on a lipid basis rather than wet weight [28]. In general, the POP concentrations in adipose tissue and blood in humans and rats were proportional to the percent lipids in their respective tissues [30] and are independent of octanol/water partition coefficients. This implies that the lipophilic compounds in the blood plasma should reflect those of the body burden. During periods of growth, however, the plasma may not reflect the body burden particularly well, as the blood will be biased towards the deposition or liberation of POPs to or from the long-term lipid stores.

No tissue type or age class or taxonomic group is an "optimal" target for contaminant monitoring of wildlife. Rather, the species selected, age and tissue type (or a myriad of other considerations) should reflect the questions that are being asked, and each selection has its own advantages and disadvantages. Whatever the purpose of the wildlife monitoring programme, researchers should understand some of the issues that affect the interpretation of body burdens.

Monitoring programmes were generally introduced either when legacy POPs were still in use or were at very high exposure levels in the environment, and they typically focused on PCBs, dioxin and dioxin-like compounds, organochlorine pesticides and mercury [210]. As stated earlier, concentrations of POPs have decreased, sometimes dramatically so, since the 1970s [156, 166, 174, 176], largely

due to regulations and restrictions or bans on the production or uses of these compounds [210]. Fiscal reality has placed pressure on many of these monitoring programmes, and there has been a movement to reduce the scale or to cut monitoring programmes of POPs entirely. Nonetheless, with some exceptions (PFOS; [211]), it is generally legacy compounds such as PCBs that trigger exceedances of thresholds, either for the consumption of fish by humans [212] or the consumption of fish by wildlife (e.g. fish flesh criteria for piscivorous wildlife; [213]), or the consumption of wildlife by other wildlife [214]. Monitoring programmes provide sound science-based information that can be used not just by scientists but also by policy makers and risk managers. Ultimately, the future of monitoring programmes of legacy POPs depend on the tradeoffs between the necessity of the monitoring or surveillance of newer, emerging compounds and the continual need, or lack thereof, of environmental data on legacy compounds.

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Dioxins and Dioxin-Like Compounds in Food and Feed

Martin Rose

Abstract Dioxins (PCDD/Fs) and polychlorinated biphenyls (PCBs) are environmentally persistent organic pollutants (POPs) that are associated with human health effects. These substances persist for long periods of time in the environment and accumulate and pass from one species to the next through the food chain. Human exposure to POPs is mainly through contaminated foods, and certain cultures or individuals whose diets include large amounts of fish or wild foods that are high in fat are particularly at risk of high exposure. In addition to the PCDD/Fs and PCBs, several other classes of contaminants behave similarly and share some common environmental fate and toxicological characteristics. These include well-known legacy contaminants such as DDT and certain chlorinated pesticides, as well as several new or emerging classes of persistent, bioaccumulative and toxic (PBT) substances. To make reliable estimates of human dietary exposure, it is important to have a robust sampling and analysis methodology and have sound knowledge about dietary preferences and food consumption patterns. In general, the highest levels of dioxins, PCBs and other PBTs are typically found globally in the fatty tissues and livers of fish and wildlife that occupy higher tropic levels in aquatic and terrestrial food chains. Toddlers and young children are typically at risk of higher exposure than adults because of their lower body weights and relatively higher food intakes. Dioxin and PCB levels in the food supply are generally decreasing, but high levels persist in some regions due to legacy contamination from industrial activity, the consequences of wartime use of defoliants, global cycling to the northern polar regions and isolated food contamination incidents. There is evidence, however, that

The views expressed in this article are my own.

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exposure to new or emerging PBTs with dioxin-like characteristics such as certain brominated substances could be increasing in importance.

Keywords Dietary exposure, Dioxins, Feed, Food, Food intake, PBTs

Contents

1 Introduction

PCDDs and PCDFs ("dioxins") are defined under the Stockholm Convention as persistent organic pollutants (POPs). They are widely dispersed in the environment and bioaccumulate in the food chain. They are ubiquitously present in human tissues even when there is no history of occupational or accidental exposure. Although exposure could occur through inhalation of air, dermal absorption, consumption of drinking water and consumption of food, there is no doubt that the latter is by far the major route of exposure for the majority of the population.

There are 75 possible dioxin congeners and 135 possible furan congeners. The dioxin and furan congeners thought to be most toxic to humans are the seven dioxins and ten furans with a particular pattern of chlorines known as the 2,3,7,8-congeners. With the exception of the 2,3,7,8-congeners, few of the dioxins and furans are persistent and readily absorbed and retained in animal tissues, and most pose low risks to human health. The 2,3,7,8-congeners are regarded as significantly toxic, highly lipophilic and are, thus, the focus of health and environmental research.

1.1 Evolution of Toxic Equivalency Factors

Because of the need to assess the risk from mixtures of many different individual PCDD/Fs released from a wide variety of anthropogenic sources, scientists and regulatory agencies have adopted an approach that assigns relative toxic potency factors to the 2,3,7,8-congeners, based on a comparison to the potency of the most toxic 2,3,7,8-tetrachlorodibenzodioxin (TCDD) [1]. Each chemical is assigned a toxic equivalency factor (TEF). The total toxic equivalency (TEQ) of a PCDD/F mixture is the sum of the product of the TEF and concentration for each 2,3,7,8-congener in the mixture. There is some variation in the terminology used by different authors and researchers must take care to examine data sets closely when compiling data from different studies; for example, while total TEQ, summed TEQ and Σ TEQ are self-explanatory, the composition of 2,3,7,8-congeners included in the TEQ or the TEF scheme used to calculate the TEQ may be different between studies.

Although consideration of total TEQs is essential, it is regrettable that many PCDD/F data sets reported in the scientific literature do not include the underlying 2,3,7,8-congener test results. As discussed below, the interpretation of PCDD/F environmental data reported as TEQ is greatly enhanced by inspection of the underlying congener-specific data, especially when data from different laboratories are compared. The contribution of specific congeners to the total is also of great value for source identification.

Though TEF schemes have evolved over the past nearly 30 years, there remain some uncertainties concerning the accuracy with which TEF values reflect actual effects on humans. It should be noted that TEF values are derived only to the nearest one-half order of magnitude, and thus the range within which the "true" TEF lies is right skewed, from ½ to 5 times its stated value (depending on which TEF scheme is used). This is a source of uncertainty that is specific to treatment of data expressed on a TEQ basis, and should be considered when conducting any risk assessment.

1.1.1 PCDDs and PCDFs

During the 1980s, several different TEF schemes were proposed and debated in the scientific community. The first TEF scheme to be widely adopted by regulatory authorities and scientists, the International Toxic Equivalency Factors (I-TEFs) for PCDD/Fs, was set in 1990 [2]. The World Health Organization proposed a TEF scheme in 1997 (WHO-TEFs, and sometimes referred to as WHO97 TEFs or WHO98 TEFs), and modified the scheme in 2005 [1, 3]. To date, the WHO-TEF 2005 scheme is widely accepted by most authorities and scientists. Notable changes in the WHO-TEF scheme include a higher TEF for 1,2,3,7,8-PeCDD (1.0, as opposed to 0.5 in the I-TEF scheme) and tenfold lower TEFs for OCDD and OCDF (from 0.001 to 0.0001). The net effect of these changes for food monitoring programs was a widespread 15–20% increase in the calculated total TEQ level of PCDD/Fs in many foods. Several smaller changes were made to the WHO 2005

Table 1 Toxicity equivalency factors (TEFs) proposed by WHO showing the changes between the 1998 and 2005 schemes

Compound	WHO 1998 TEF	WHO 2005 TEF[a]
Chlorinated dibenzo-p-dioxins		
1,2,3,7,8-PeCDD	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.0001	**0.0003**
Chlorinated dibenzofurans		
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	**0.03**
2,3,4,7,8-PeCDF	0.5	**0.3**
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,6,7,8,9-HpCDF	0.01	0.01
OCDF	0.0001	**0.0003**
Non-ortho substituted PCBs		
PCB 77	0.0001	0.0001
PCB 81	0.0001	**0.0003**
PCB 126	0.1	0.1
PCB 169	0.01	**0.03**
Mono-ortho substituted PCBs		
105	0.0001	**0.00003**
114	0.0005	**0.00003**
118	0.0001	**0.00003**
123	0.0001	**0.00003**
156	0.0005	**0.00003**
157	0.0005	**0.00003**
167	0.00001	**0.00003**
189	0.0001	**0.00003**

[a]Bold values indicate a change in TEF value

scheme, including changing TEF values with 0.5–0.3 to reflect the fact that the derivation of the scheme used a log scale (Table 1).

1.1.2 Polychlorinated Biphenyls

WHO also established TEFs for polychlorinated biphenyls (PCBs) that are known or strongly suspected to bind to the Ah-receptor and elicit biochemical behaviours

and toxic responses similar to the 2,3,7,8-dioxins. In 1994, TEFs were set for three non-*ortho* PCBs (IUPAC Nos. 77, 126 and 169), eight mono-*ortho* PCBs (105, 114, 118, 123, 156, 157, 167 and 189) and two di-*ortho* PCBs (170 and 180) [4]. In 1997, WHO adopted a PCB TEF scheme [3] that eliminated consideration of PCBs 170 and 180 as dioxin-like, added PCB 81, and reduced the TEF for non-*ortho* PCB 77 by a factor of 5. For most food monitoring programmes, the changes made a negligible difference to the total TEQ attributable to PCBs.

The term co-planar PCB is often used to refer to three non-*ortho* PCB compounds, and sometimes to eight mono-*ortho* PCBs. In many food monitoring programmes, and particularly those focused on fish products, the TEQ contribution from PCBs may equal or exceed the TEQ contribution attributable to PCDD/Fs (see below). Consequently, it is of paramount importance when comparing or interpreting data expressed as TEQs to determine whether or not co-planar PCBs are included in the total TEQ.

1.2 Legacy and Emerging Contaminants

In addition to the PCDD/Fs and PCBs, there are several other classes of contaminants that behave in a similar way and share some common environmental fate and toxicological characteristics. These include well-known legacy contaminants such as DDT and certain chlorinated pesticides, as well as several new or emerging classes of persistent, bioaccumulative and toxic (PBT) substances. The term "emerging contaminants" is used to describe new chemicals that are known or suspected to have PBT properties.

Emerging contaminants are characterised by the properties of bioaccumulative potential (i.e. they are found at higher concentrations at higher trophic levels of the food web), environmental mobility (i.e. they undergo long-range transport), persistence (i.e. they are stable and do not readily degrade in the environment) and toxicity (i.e. they are harmful to living organisms). Some substances have been placed on the Stockholm POPs registery (http://www.pops.int) – which was originally limited to 12 legacy chemicals, and in recent years extended to include several additional substances with the potential to increase further in the future.

The 12 initial POPs addressed by the Stockholm Convention include nine pesticides (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex and toxaphene); two industrial chemicals (PCBs and hexachlorobenzene); and the dioxins and furans. The nine new chemicals added to the Stockholm Convention were classified as either Annex A (elimination), B

(restriction) or C (unintentional production). The names of these chemicals and their respective Annexes are as follows:

- Alpha hexachlorocyclohexane to Annex A;
- Beta hexachlorocyclohexane to Annex A;
- Hexabromodiphenyl ether and heptabromodiphenyl ether to Annex A;
- Tetrabromodiphenyl ether and pentabromodiphenyl ether to Annex A;
- Chlordecone to Annex A;
- Hexabromobiphenyl to Annex A;
- Lindane to Annex A;
- Pentachlorobenzene to Annex A and C;
- Perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride to Annex A or B.

It is widely acknowledged that other classes of contaminants also bind to the Ah-receptor and elicit dioxin-like biochemical and toxic responses similar to the 2,3,7,8-dioxins and furans and in addition to the co-planar PCBs. These include brominated and mixed halogenated analogues of the PCDD/Fs and PCBs, and also other chemical groups such as polychlorinated (and brominated) naphthalenes and other larger halogenated polycyclic aromatic hydrocarbons (PAHs). Consequently, the assessment of health risks of exposure to dioxin-like chemicals should consider this wider group of substances. Some of these contaminants also have other biological effects, that are also important to consider. Even if these other effects are taken aside, consideration of dioxin-like toxicity using the established TEF scheme is incomplete without the inclusion of all compounds that behave with a similar biological mechanism.

2 Food as a Sample Type

Given that food is recognised as the most important source of exposure, it is important to estimate exposure from this source, and to be able to ensure consumer safety by effective food control measures. Therefore, robust chemical testing methods are important to measure these contaminants in food, in order to make reliable estimates of dietary exposure.

2.1 Analytical Methodology: Standard Methods, Criteria Based Approach and Research Methods

Several approaches used to provide confidence in analytical data include: (1) the use of Standard Methods, (2) the use of analytical criteria that describe the performance of a method and (3) consideration of "fitness for purpose" based on

measurement uncertainty. The importance and difference in these approaches was discussed by Rose et al. [5] and is summarised below.

Due to the complexity of dioxins analysis, and the low levels of measurement that are needed, standard methods for food testing have been historically somewhat limited. The US EPA method for environmental samples employs stable isotope dilution and gas chromatography with high resolution mass spectrometry and has been used as a basis for methodology for many years. A standard method specifically for animal feed, but also widely used for food, was produced in Europe by CEN in 2012 (CEN 16215). This bears many similarities to the US EPA method and is also based on stable isotope dilution and gas chromatography with high resolution mass spectrometry.

Whilst a standard method can be expensive and time consuming to produce, it can provide consensus among interested parties and stakeholders (e.g. vendors, buyers, enforcement agencies, academia, etc.). Due to the amount of effort and the number of partners involved in its production and because of its wide acceptance, standard methods are often seen as best practice in the sector to which they are applied; they can support free trade, and reduce costs.

In the European Union (EU), there is a "hierarchy" of analysis methods used for official control purposes. Sampling and analysis methods used in the context of official controls need to comply with relevant European Community rules or, (a) if no such rules exist, with internationally recognised rules or protocols (e.g. those that the European Committee for Standardisation (CEN) has accepted or those agreed in national legislation), or, (b) in the absence of the above, with other methods fit for the intended purpose or developed in accordance with scientific protocols.

The practical advantages associated with the use of standard methods are:

- They are generally methods that are based on widely accepted principles with sufficient validation data and proven transferability to other laboratories.
- They give a clear description with all details including calibration and calculation.
- They have been agreed by the interested parties and stakeholders.
- Standard methods are usually designed to use equipment and techniques that can be accessed by as wide a range of laboratories as possible.
- Accreditation bodies would only need to review a standard method once in detail.
- Many standard methods are available in more than one language (CEN produces standards in English, German and French).
- They are particularly useful if it is necessary to demonstrate to, and gain agreement from, all stakeholders that actions based on the results of analytical tests are a necessary protection for consumers rather than potential barrier to free trade.
- They are also a starting point for new laboratories, for laboratories involved with a wide range of functions where a variety of analyses are undertaken.

There are some disadvantages associated with standardisation and standard methods. For example, the process of converting a good analytical method into a *standard* method can be laborious. The basis for any method used to enforce food safety regulatory requirements is providing evidence that a method delivers valid results. A newly developed and single-laboratory validated method will normally be subject to formal validation by collaborative laboratory trials, usually organised by the method provider or sometimes by a standards body such as the Association of Official Analytical Chemists (AOAC), CEN or some other organisation using international protocols. The performance data from such an exercise can be used to give a firmer indication of fitness for purpose across a number of laboratories. Valid sets of results from at least eight laboratories are usually required for such a trial to give sufficient data to calculate repeatability and reproducibility. The method may then go through a process of being considered, approved and eventually issued as a standard. The process of converting a method that is considered to have demonstrated sufficiently good performance into as a *standard method* will usually take at least 2 years.

There are a number of ways in which the performance of methods may be described which may be particularly useful for different stakeholders. Broadly, method performance might be described using: analytical performance "criteria" such as those traditionally used by analysts (the criteria approach), measurement uncertainty as applied in analytical chemistry since around the turn of the century (the standard uncertainty approach) or by evaluating the consequences of measurement uncertainty for stakeholders (the uncertainty profile approach) [5].

2.2 Samples and Representativeness

The most accurate laboratory analysis is only representative of the sample taken for analysis. How that sample informs the larger food supply depends on both the quality and the intent of the sampling scheme. For use in estimation of human intake, sampling should be designed to take account of many factors such as the proportion of each food that is imported, and variation in the food quality among different countries of origin. Seasonal variation, for example, may occur when supplies available to the consumer vary at different times of year, or when favoured food imports are prevalent at only certain times, or if food has been stored for longer periods to supply markets during out of season times. Regional variation, on the other hand, refers to local food production affected by differences in climate or by local pollution sources or urbanisation. Food prepared ("take-away" meals) or eaten outside the home (in restaurants, for example) also needs to be considered.

Sampling to assess compliance with limits, whether statutory or "guidelines", may not be appropriate for estimating average human intakes. For example, compliance monitoring may focus on domestic food production and exclude imports, or aim for broad geographical coverage without weighting by food production statistics to focus more on those regions where most of the food of a given type is

produced. Any indication of localised contamination usually leads to more intensive sampling; small but significantly contaminated locations may make a disproportionately large contribution to "average" concentrations.

For most foodstuffs, achievement of a representative result inescapably necessitates coverage of a large number of samples. However, due to the cost and difficulty of analysis, most of the older studies from the late 1980s and early 1990s, and some completed more recently, were based on rather limited numbers of samples, sometimes even on single food products. To overcome this issue, a pool of samples can be used to form composites representing a specific category of foodstuff. This approach has been used in several national studies such as in Finland, the Netherlands, New Zealand and the UK [6–8]. The use of composite samples is a cost-effective way of obtaining robust measures of average concentrations but it does not furnish any information on the width and shape of the distribution of concentrations in the individual samples. It may also place great reliance on single analyses.

Total diet study (TDS) schemes have often been used as the source of samples. This concept has been used for many years, and has been adapted and modified over time in some countries [6–8]. In the UK, TDS in place after 1983 [8] involves a total of 121 categories of food and drink purchased fortnightly from 24 randomly selected locations representative of the UK. Samples are prepared and cooked for consumption and combined into composite samples representing 20 defined food groups. The quantity and relative proportions of the foods that make up each composite were formerly based on data from the National Food Survey (a continuous survey which provides information on the types and quantities of foods purchased by households). This survey has been replaced in recent years by the Annual report on household purchases of food and drink, published as "Family food" and available from https://www.gov.uk/government/collections/family-food-statistics. TDS schemes vary considerably in their geographic range, in the number of individual samples taken and in the number and timing of samplings which vary from a single occasion to repeated sampling over a year.

Differences in food classification can also lead to a lack of comparability between studies. For instance, in the UK TDS, the meat group is not segregated by animal species and includes beef, mutton and pork. In many studies some classes, such as meat products, fruit products and cereals, are differently defined and not comparable. Cereal, for example, may refer simply to grain and flour, or could include cereal products encompassing breads, cakes and pastries prepared with animal fats, and sometimes various "breakfast foods". This led, in 2011, to the European Food Safety Authority (EFSA) together with the WHO and The Food and Agriculture Organisation of the United Nations (FAO), producing guidance on how to conduct a TDS.

2.3 Expression of Results

Different food products are conventionally reported either on a fat weight basis, a whole weight basis (typically for low fat foods), or on a 12% standardised moisture content or whole weight basis for some dried foods and more typically for dried animal feeds. In terms of units, convention is usually to adopt picogramme/gramme (pg/g) when reporting results for dioxins and DL-PCBs, and nanogramme/gramme (ng/g) or microgramme/kilogramme (μg/kg) for NDL-PCBs.

The consumption of food of animal origin is generally the main route of human exposure to dioxins and PCBs. Due to the accumulation in the fat of the food of animal origin, maximum levels (MLs) for dioxins and DL-PCBs are generally expressed on a fat basis. The MLs for muscle meat of fish, fishery products and products, however, are often expressed on a whole weight basis because of the wide range of fat content that is found in different fish species. Because ingested dioxins and PCBs are stored and concentrated in the fatty tissue of the fish, species with a low fat content such as cod with around 0.4% fat could have extremely high levels if the concentrations of the lipophilic compounds were expressed on a fat basis.

As a consequence, a considerable number of low fat fish species would exceed MLs on a fat basis, while fish species with high fat content of 20% and more, such as eel or herring, would seem favourable because of the greater dilution of the lipophilic compounds in the higher fat amount. However, it is not the isolated fat but the muscle meat of fish that is consumed and thus the maximum levels based on a fresh weight basis allow a better estimation of the actual human exposure and potential health impacts. For purposes of exposure estimates and risk assessment, it may make more sense to express all results on a whole weight basis [9].

3 Reported Levels of Dioxins and PCBs in Different Types of Food and Feed

The largest published collection of quality tested data on dioxins and PCBs is likely held by the European Food Safety Authority (EFSA), as reported in 2010 [9] and updated in 2012 [10]. The 2012 update summarised data reported to EFSA as a result of national monitoring programmes within the EU. National monitoring programmes have been conducted on a regular basis as a result of the EC strategy for dioxins and PCBs adopted by the Commission on 24 October 2001 following a major food contamination incident involving dioxins and PCBs in Belgium a few years earlier. The EC strategy was designed to address measures to limit or to eliminate environmental releases through source-directed measures and to decrease the presence of dioxins and PCBs in food and feed.

Maximum levels (MLs) for the sum of dioxins, the sum of dioxins and DL-PCBs and the sum of 6 NDL-PCB indicators in food and feed are specified in Commission Regulation (EC) No 1881/2006 setting MLs for certain contaminants in foodstuffs, as amended by the Commission Regulation (EU) No 1259/2011, and in the

Directive 2002/32/EC on undesirable substances in animal feed, as amended by Commission Regulation (EU) No 277/2012. The MLs are expressed as TEQ WHO (2005) [1] for dioxins and DL-PCBs and on the direct sum of the 6 NDL-PCB indicators.

In addition to MLs, action levels (ALs) were established at a lower level. Exceedance of the AL would not lead to withdrawal of food from the market, but would trigger investigation as to the source of the contamination and action to reduce any releases of PCDD and/or PCBs into the environment. Maximum and action levels were calculated on the assumption that all values of the different congeners below the limit of quantification (LOQ) are equal to the LOQ, which corresponds to an upper bound (UB) concentration. Levels for foodstuffs of terrestrial animal origin and marine oils are given on a fat (lipid) weight (lw) basis. For the products of aquatic origin, except marine oil, and products of plant origin, they are expressed on a whole weight basis (ww), whereas for feed they are expressed on 88% dry weight (dw) basis. For foods containing less than 2% fat, the maximum level is expressed on a product basis, defined as the maximum level expressed on fat for that food multiplied by 0.02. The action levels are not applicable for foodstuffs containing less than 2% fat [10].

Results from national monitoring programs on the presence of dioxins and PCBs in food and feed have been reported on a regular basis to the Commission. In 2010, following a request of the Commission, EFSA produced a first compilation of the results of the monitoring of dioxins and PCBs in food and feed, which resulted in two reports (EFSA, [9, 10]). Levels of dioxins and DL-PCBs from 7,270 samples, and non-dioxin-like PCBs (NDL-PCBs) from 12,563 samples collected between 1995 and 2008 from 21 EU Member States, Iceland and Norway were compiled.

The highest levels of dioxins and DL-PCBs were observed in liver products from both aquatic and terrestrial animals (on average, 32.6 pg TEQ WHO98/g ww and 5.7 pg TEQ WHO98/g lw, respectively), on eels muscle (on average 6.7 pg TEQ WHO98/g ww) and in fish oil for animal feeding (on average 10.0 pg TEQ WHO98/g dw). The percentage of results exceeding the maximum level for dioxins and DL-PCBs was on average 8% with a further 4% exceeding the action levels. The highest levels of NDL-PCBs were observed in products derived from aquatic animals (from on average 23.3 μg/kg ww for muscle from fish other than eels to 223 μg/kg ww for eel muscle), followed by products derived from terrestrial animals (from on average 1.04 μg/kg lw for pig fat to 16.7 μg/kg lw for egg products) and feed for fur animals, pets and fish (11.1 μg/kg dw). A detailed analysis of the contamination profiles revealed that PCDD/Fs represented between 30 and 74% of the total TEQ depending on the food or feed group, while mono-*ortho* PCBs represented between 15 and 45% of the DL-PCBs. For NDL-PCBs, PCB-153 and PCB-138 together consistently comprised at least 50% of the overall sum of the six indicator PCBs in each food group. Both reports recommended to pursue testing dioxins and PCBs in food and feed on a random basis and to improve the reporting of the sampling strategy at the sample level.

The data compilation gave 16,238 and 32,984 samples being checked for compliance to analytical performance criteria defined for dioxins and DL-PCBs,

and NDL-PCBs, respectively. Many of the results submitted were excluded since they did not meet required analytical performance criteria [10].

3.1 Dealing with Results Below the Limit of Detection

According to the WHO guidelines on censorship of data (GEMS/Food-EURO, 1995), when more than 40% of the results were quantified at the food and food group levels, the average contamination level was estimated by considering the non-detected/quantified results at half of their respective LOD/LOQ (middle bound approach). In the other cases, the average contamination level was estimated at the lower and upper bound values and both results were reported. The contamination levels corresponding to vegetable oils and fats, and products from terrestrial animals were expressed on a fat content basis whereas the contamination levels of other foods were expressed on a whole weight basis.

3.2 Contamination Levels Across Food Groups

Using methods of analysis that have been generally applied for the last 10 years or so, it is possible to measure at least some PCDD/Fs and PCB congeners in most foods when there is sufficient sample available.

The discussion below is based upon the data presented in the EFSA report mentioned above [10]. Highest levels are typically found in fish liver and liver from terrestrial animals, followed by muscle meat from eel. In these food types, it is usual to find levels above 10 pg $TEQWHO_{05}$/g ww for fish products and lw for liver from terrestrial animals. Food types that typically have levels lower than that limit of 10, but above 1 pg $TEQWHO_{05}$/g lw, are all foods based on animal products and include "Hen eggs and eggs products", "Meat from fish other than eel", "Meat from bovine animal and sheep", "Raw milk and milk products", "Marine oil" and "Other food products".

"Meat from poultry" contain on average low levels of dioxins and DL-PCBs (<1 $TEQWHO_{05}$/glw), but high levels are sometimes found in a few samples. The "Other food products" group comprised quite heterogeneous food items. Among them, in accordance with previous observations, the foods of terrestrial animal origin – "Edible offal from farmed animals", "Game mammals and birds" and "Livestock meat from other animals than pig, bovine and sheep" – contained the highest levels of dioxins and DL-PCBs. "Composite foods" and "Products of nutritional use" also contained on average more than 1 pg TEQWHO2005/gww dioxins and DL-PCBs. Some supplements based on marine oils were found among the products for nutritional use, which may explain their level of contamination.

The quantification rate is generally lower for the indicator PCBs than for dioxins and DL-PCBs, but more variable between the food groups. Whereas it was higher in

"Fat of poultry", "Fish liver and derived products", "Liver from terrestrial animals", "Muscle meat from fishes other than eels" and "Raw milk and dairy products", it is lower for "Meat pigs", "Meat poultry" and "Other food products". Two food groups were clearly distinct from the others: "Fish liver and derived products" and "Muscle meat from eels" with mean levels for the sum of the six indicators higher than 200 μg/kg ww. Five groups had mean levels between 10 and 30 μg/kg lw/ww: "Meat poultry", "Other food products", "Muscle from fishes other than eels", "Liver from terrestrial animal" and "Hen eggs and egg products". The high levels observed in the "Other food products" are explained by the "Products for special nutritional use", "Unspecified fish and seafood products", "Game mammals and birds" and "Transformed and unspecified meat products", which contained on average more than 20 μg/kglw/ww of the six NDL-PCB indicators. A high variability was noticed for "Vegetable oils and fats" with a few samples highly contaminated. The quantification rate of the six NDL-PCB indicators varied considerably between the feed groups. Whereas it was higher than 95% for "Animal fat" and "Fish oil", it didn't exceed 20% for "Additives binders and anti-caking agents". "Fish oil" was the most contaminated feed, followed by "Feed for fur animals, pets and fish", "Other feed additives" and "Fish, other aquatic animals and their products", with average levels higher than 5 μg/kgdw. Similarly to the food group "Vegetable oils and fats", a high variability of contamination was observed for the feed group "Vegetable oils and their by-products".

3.3 Contribution of the Individual/Group of Congeners

Figures 1 and 2, and the figures and discussion below are taken from the EFSA report [10]. Figure 1 illustrates the relative contribution of PCDDs, PCDFs, mono-*ortho* PCBs and non-*ortho* PCBs, expressed in TEQWHO05, to the lower and upper bound estimates of the total TEQ WHO05 of dioxins and DL-PCBs in food and feed. Overall, the main contributors were the non-*ortho* PCBs, which gave between 21.0 and 74.9% of the total TEQ WHO05 of dioxins and DL-PCBs in food, followed by the PCDDs and PCDFs which together contributed between 12.4 and 73.2% of the total TEQWHO05. The mono-*ortho* PCBs contributed less than 12% of the total TEQWHO05. When looking at the most contaminated samples, the relative contribution of the non-*ortho* PCBs increased from 34.2 to 86.1% depending on the food group.

The food groups with the highest levels of non-*ortho* PCBs (more than half the total contamination) were products from aquatic animals and from ruminants. The contamination profile of the feed groups was similar to what was observed in food, except for four of the feed groups. In the "Additives binders and anti-caking agents", the PCDDs represented the major contributor with more than 80% of the total TEQWHO05. In the "Additives compounds of trace element", "Other feed additives" and "Feed materials of plant origin, oils excluded", the PCDFs were the major contributor with between 29.0 and 92.4% of the total TEQWHO05. As observed in the food groups, the most contaminated feed group samples had an

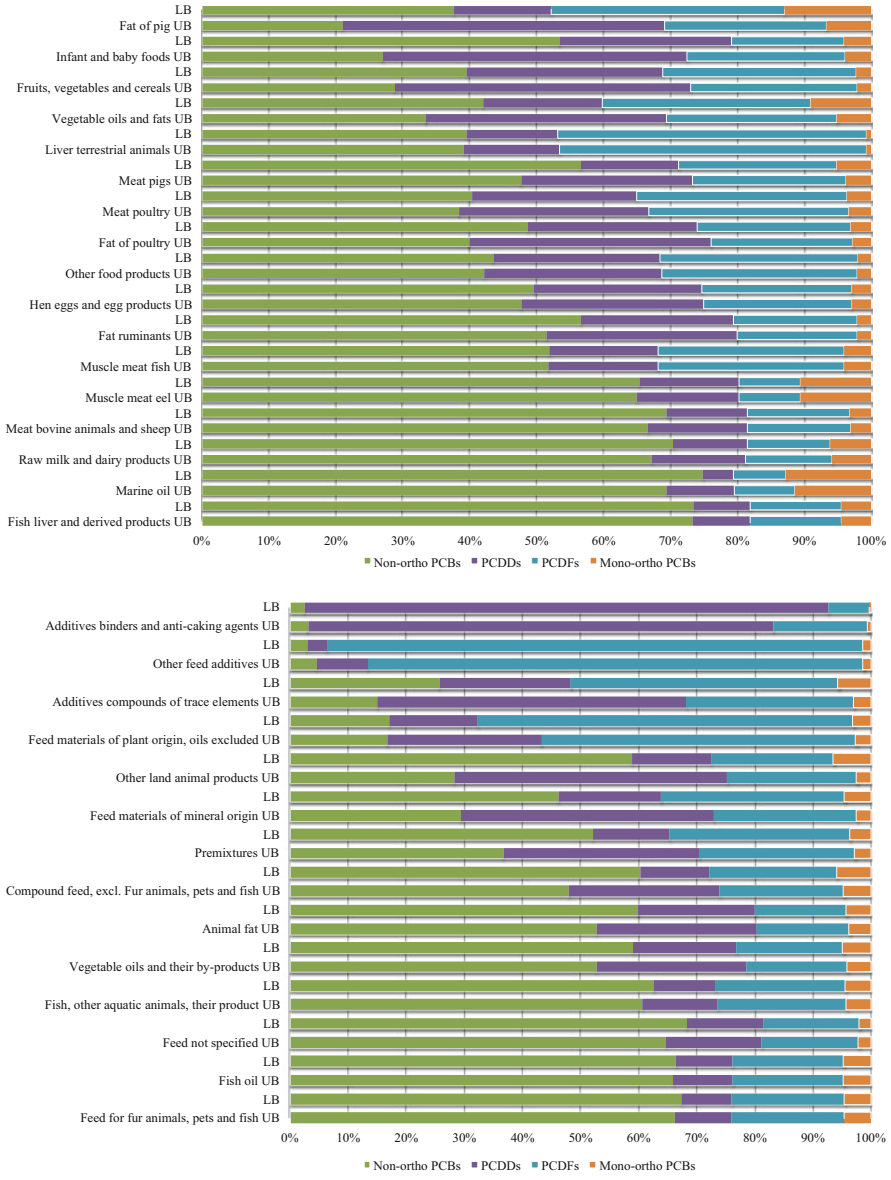

Fig. 1 Relative contribution of PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs to the total TEQWHO05 of dioxins and DL-PCBs in food (*top*) and feed (*bottom*) at lower (LB) and upper bound (UB) concentrations. *Source*: European Food Safety Authority (EFSA, [10])

increased relative contribution of non-*ortho* PCBs to the total TEQWHO05, up to two times higher than the average contribution.

Figure 2 illustrates the average relative contribution of each individual indicator to the sum of the six NDL-PCB indicators for the lower and upper bound estimates in food and feed. Except for “Fruit, vegetables and cereals”, the major contributor

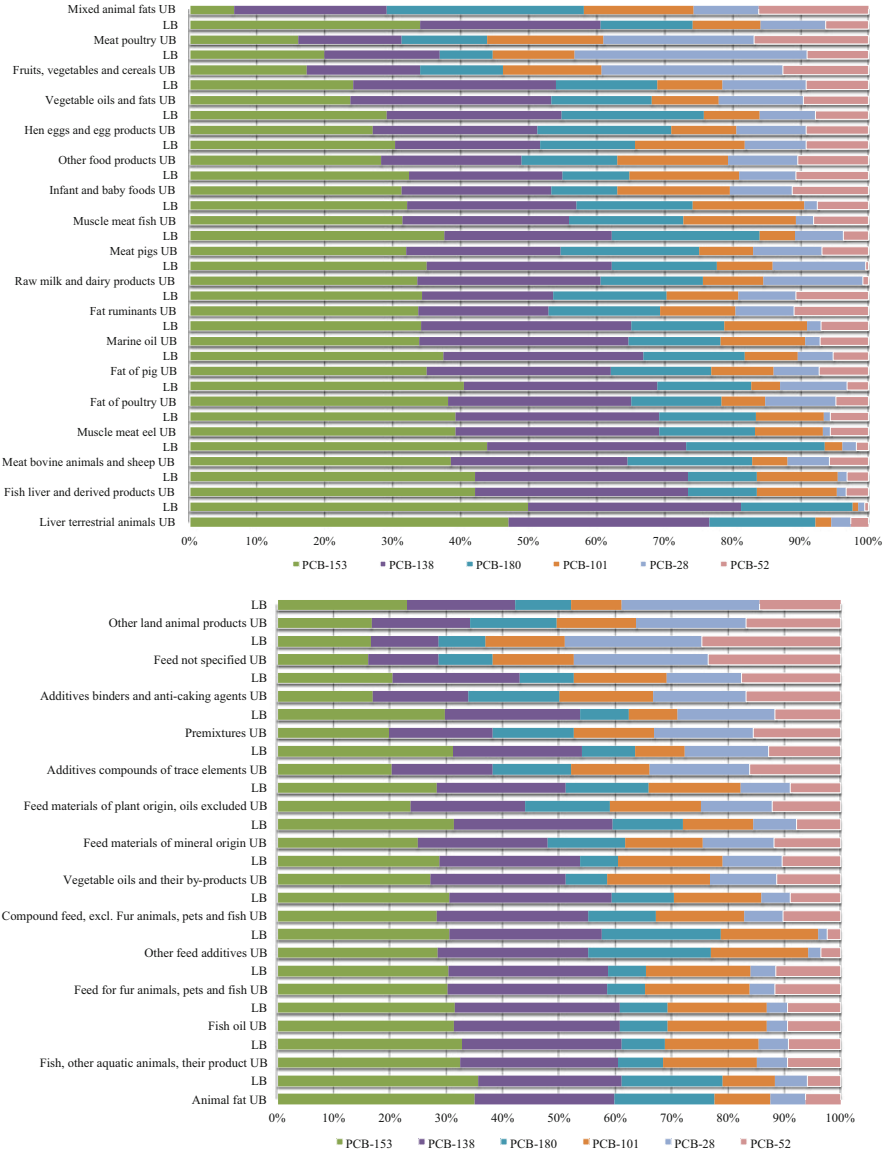

Fig. 2 Relative contribution of each indicator to the total level of the six NDL-PCB indicators in food (*top*) and feed (*bottom*) at lower (LB) and upper bound (UB) concentrations. *Source*: European Food Safety Authority (EFSA, [10])

was PCB-153, followed by PCB-138 and PCB-180, which together contributed between 43.7 and 97.8% of the sum of the 6 NDL-PCB indicators in food. The contribution of the three other NDL-PCBs varied between 0.3 and 34.3% according to the food group. In "Fruit, vegetables and cereals", the major contributor was

PCB-128, which represented around one third of the sum, the five other NDL-PCBs were almost equally distributed. The most contaminated samples had similar profiles of contamination.

In feed, the major contributor was also PCB-153 (between 16 and 36% of the sum of the six indicator PCBs), closely followed by PCB-138 (between 12 and 29%). The contributions of the four other PCBs varied more greatly according to the feed groups. PCB-180 and PCB-101 contributed at least to 6% and up to 20% of the total level represented by the six indicator PCBs. PCB-28 and PCB-52 contributed at least to 2%, but could represent up to 28 and 24% of the sum of the six indicator PCBs, respectively. The most contaminated samples followed a similar pattern of contamination, consistent with those described in the scientific literature. A more detailed analysis broken down to individual congeners can be used to give further information, for example, can be used to help identify the source of the contamination. Patterns of dioxins produced as a result of incineration have a different 'fingerprint' to the pattern where dioxins are a result of by-products in the production of organochlorine chemicals, or as a result of PCB contamination, etc. Congener patterns may even be used to identify whether or not a contaminated food sample is a result of a specific incident (see later).

3.4 Time Trend Analysis

There are established software packages designed to detect and estimate trends in atmospheric and precipitation concentrations such as the MS Excel® application "MAKESENS", originally developed by the Finnish Meteorological Institute [11]. This application performs two types of statistical analysis. Firstly, the presence of a monotonic increasing or decreasing trend is tested with the non-parametric Mann–Kendall test. Secondly, the slope of a linear trend is estimated with non-parametric Sen's method. The information should be available for a minimum of 4 years (which can be non-consecutive) for the tests to be applied and 10 years for confidence intervals around the slope estimate to be characterised. Missing values are allowed and the data need not be normally distributed.

Time trends can be used to observe changes in contamination load of specific food types, e.g. "fish", or "milk", or they can be used to estimate changes in consumer intake. For the latter, it is important to realise that dietary habits change with time. This means that trends in dietary exposure cannot be estimated by comparison of levels of contamination of different food items alone. Changes in dietary consumption need to be reflected in exposure estimates.

Given the size of the database, the EFSA data collection made it possible to undertake a fairly robust time trend analysis for hen eggs and egg products, fish (other than eels) and for milk and dairy products [10].

An overall decreasing trend was observed for the median level of the sum of dioxins and DL-PCBs and of the sum of the six NDL-PCB indicators over the years.

When taking the whole time series into the account, this decrease was statistically significant with the Mann–Kendall trend test in:

- "Raw milk and dairy products" for the sum of dioxins and DL-PCBs: 56% reduction in 10 years with a starting median value estimated at 1.17 pg TEQWHO05/glw,
- "Raw milk and dairy products" for the six NDL-PCB indicators: 64% reduction in 15 years with a starting median value estimated at 9.95 μg/kglw,
- "Muscle meat from fishes other than eels" for the sum of dioxins and DL-PCBs: 98% reduction in 10 years with a starting median value estimated at 3.05 pg TEQWHO05/gww.

It could not be excluded that the heterogeneity in the foods constituting the groups, in the countries of origin covered and targeting strategies between the years, had influenced the observed trends, especially the estimation of the rates of decrease. For example, concerning the sum of the six NDL-PCB indicators in raw milk and dairy products, the observation outside the 99% confidence interval for year 2009 appeared to be driven by samples from Germany and Czech Republic. These samples represented 62% of the data set available that year and were on average three times more contaminated than those from the other countries. The analysis could not clearly attribute the reason for the possible change since it can be due to risk management measures, but also it may have been due to improvements throughout the years in both analytical methods and/or sampling designs of the monitoring programs.

4 Specific Issues Relating to Some Food Types

4.1 Liver

Liver, especially from sheep or venison, contains higher levels of PCDD/Fs on a fat weight basis than other tissues from the same animal. This difference is not associated with poor husbandry practice, but more likely is due to the way that the animal metabolises these compounds and in particular due to sequestration in the liver. The reason that sheep may show a larger effect than other animal such as cattle could be due to the way they feed. For animals such as sheep, the involuntary intake of soil can occur through particles deposited on vegetables or directly when feeding on pasture herbage close to ground surface. Cattle normally feed on vegetation above 5–10 cm from the ground surface; sheep are anatomically able to nip closer to ground surface. The grazing surface is generally wider for sheep as flocks tend to change pasture fields more frequently than cattle, thus increasing the probability of coming into contact with land with variable levels of contamination [12–14].

4.2 Eggs

Eggs from caged birds have been shown to be significantly less contaminated than those coming from free range outdoor production. This is possibly due to the availability of soil and grit which the animals peck [15].

4.3 Fish

Farmed salmon and trout have been reported to contain higher amounts of dioxins and PCBs than wild varieties, but some of the early studies compared farmed varieties from one part of the world with wild varieties from another [16, 17]. The EFSA database showed that for a more substantial data set of fish consumed within Europe, farmed are generally less contaminated than wild caught salmon and trout for both dioxins, DL-PCBs, and the six NDL-PCB indicators. This is likely to be a result of the commercial feed used for farmed fish which contains lower levels of contamination compared to the largely fish based diet that is consumed by wild fish.

Herring, salmon and trout from the Baltic region are known to be significantly more contaminated by dioxins, DL-PCBs and by the six NDL-PCB indicators than fish from other regions. These countries are allowed "derogation" from EU regulations as long as there is an education campaign to inform consumers about limiting consumption. Part of the reason for this is that oily fish provide an important source of vitamin D which is especially important for consumers in Northern European countries where levels of light can be particularly low at some times of the year.

5 Dietary Exposure

5.1 Problems When Compiling and Using Reported Data to Estimate Exposure

Analysis of food (and feed) for dioxins is still expensive, and as such many samples that are included in surveys or sampling strategies are a result of risk based activities. This means that samples collected will be weighted to include more "high level" samples than would be included if sampling plans were of random design. For example, more samples are taken from near known dioxins "hot spots" (e.g. known sources of pollution) or as a result of intelligence – if it is known that a certain commodity from a specific location has been found to exceed limits, then samples of this type are more likely to feature in follow-up exercises. As a result, precaution is needed when using available data to calculate average population

exposure, or time trend analysis, etc. Compiling data from various sources can also result in statistical difficulties. There will be differences in limits of detection, reporting formats and different descriptions for similar food items.

5.2 Exposure Estimates

Exposure is a function of both the levels of contamination of a particular food type combined with the amount of that food type that is consumed. The population group with the greatest exposure is usually breast-fed infants. This is due to the relatively large amounts of dioxins and PCBs found in human milk combined with the low body weight of the infants. Nevertheless, advice is that breast feeding is best due to the overriding beneficial effects that are associated with the practice plus the fact that the infants are gaining weight so rapidly that the kinetics associated with deposition of contaminant load are different for this group.

Excluding breast-fed infants, the EFSA [10] report identified toddlers and other children as the most exposed groups, with an average exposure for the sum of dioxins and DL-PCBs of between 1.08 and 2.54 pg TEQWHO05/kg b.w. per day and 95th percentile exposure between 2.6 and 9.9 pg TEQWHO05/kg b.w. per day. Consequently, depending on the population group, between 7 and 52.9% of the individuals would have an exposure higher than the tolerable weekly intake (TWI) of 14 pg TEQWHO05/kg b.w., corresponding with a value of 2 pg TEQWHO05/kg b.w. per day. Other population groups have lower exposure, but the data still show that some individuals will exceed the TWI [10].

This is probably the most robust estimate available due to the large amount of data that it was based on, but it agrees with other estimates that have been reported in the scientific literature, which are summarised in the EFSA report [10].

Foods that give the greatest contribution to the TWI vary from country to country and with dietary preferences. But the foods that typically make the largest contribution include fats and oils (especially animal fats such as butter), fish and seafood (especially oily fish), meat and meat products, milk and dairy products.

When compared with historic data, there is evidence that levels are decreasing. This was especially evident prior to 2010, but the rate of decrease has been slowing since then to the point of levelling off. This is based purely on levels in different food groups and does not take into account changes in consumption habits.

6 Brominated and Mixed Bromo–Chloro-Congeners

Chlorinated dioxins, furans and biphenyls are regulated and subject to various pollution control measures which have resulted in their decrease in the environment and consequently in food over the last few decades [18]. By contrast, the use of organo-bromine compounds over a similar timeframe has been increasing, largely

as a result of their application as flame retardants. Many of these compounds have been shown to be toxic and persistent, and the use of some of the early brominated flame retardants (BFRs) such as the PBDEs has been restricted or phased out [19, 20]. Replacement BFRs often have similar properties and there is an ongoing cycle of new products and subsequent restriction.

Consequently, there is an overall increase of organo-bromine compounds released into the environment. Another common property of these compounds is that when they degrade, there is a potential for brominated dioxins and furans to be formed. If they combust alongside sources of chlorine, then there is also the possibility of formation of mixed bromo–chloro-congeners.

Currently, there is very little data available on these compounds, but the toxicological properties suggest that some congeners would have similar or even more toxic effects than their chlorinated counterparts. Occurrence data suggests that levels in foods are still lower than for the chlorinated analogues, but the concern is that whereas the chlorinated congeners are decreasing with time, these brominated and mixed halogenated congeners are likely to be increasing. There is also a problem with estimating exposure due to the complexities of analysis [21] and the very large number of congeners – 75 wholly chlorinated plus the same number of wholly brominated dioxins; 135 each for furans but 1,550 mixed dioxins and 3,050 mixed furans – giving a total of 5,020 congeners. This is combined with the fact that only a handful of analytical standards exist for these compounds making any exposure estimate very crude since it is based on measuring only a fraction of the total.

6.1 Other Compounds with Dioxin-Like Toxicity

There are other non-dioxin compounds that exhibit the same mode of toxic action as the dioxins. These can be assumed also to be additive in terms of response when an appropriate TEF is applied. These include polychlorinated naphthalenes [22–25], larger chlorinated polycyclic aromatic hydrocarbons (Cl-PAHs), and presumably their brominated and mixed halogenated analogues, and hexachlorobenzene and presumably related compounds. Since the health based guidance values are based on a TEQ, then any complete risk assessment or exposure assessment should consider all compounds that make a significant contribution to the TEQ. Going forward, it should be established whether or not these other compounds fall into that category and future monitoring schemes should be expanded to include relevant compounds. Only such a holistic approach to monitoring and food control makes sense for protecting the consumer with respect to dietary exposure to the dioxin family of chemicals.

7 Incidents

Many food chemical incidents originate from contaminated animal feed that ends up in contaminated animal production destined for human consumption. Due to their physico-chemical properties, PCDD/Fs and PCBs will increase in concentration (biomagnify) as they move to higher levels of the food chain. Dioxins and PCBs have been involved in numerous food incidents including the one that occurred in Belgium in 1999 where waste transformer oil was directly incorporated into animal feed. A more recent incident occurred in Ireland in 2008 where PCBs were used in fuel used to dry waste bread products that were used as feed ingredients.

Incidents with dioxins and PCBs have resulted in a strategy within the EU to reduce the exposure of the population to these compounds. Maximum levels were set for food and feed products and criteria were developed for the analytical methods (both confirmatory and screening) used for official control measurements. Ideally, any analysis performed with the aim of comparing the result with the legal limits should be performed according to these criteria. It should also apply to monitoring, performed to estimate human exposure and trend analysis rather than compliance with limits, since risk assessments and EU-policies rely heavily on these data.

In recent years, analytical capacity has largely increased to complement the additional testing. In line with the responsibility of producers for the safety of their products, self-control by industry has strongly increased and has played an important role in the discovery of several of the incidents. However, the increased monitoring does not seem to have resulted in a clear decrease in the levels of PCDD/Fs and PCBs reported for food and feed in the last decade. This may in part be due to a lack of follow-up when elevated levels (above action levels) are found, which would lead to a reduction of output from remaining sources. It may also be related to the sensitivity of applied methods and the data collected in databases.

Some of the key food incidents relating to dioxins and PCBs are listed in Table 2 and further information can be found from Hoogenboom et al. [26].

Table 2 Incidents with PCDD/Fs and dl-PCBs in the feed and food chain, the sources and an indication of the highest levels reported

Country	Year	Source	Highest levels[a] (food in pg TEQ/g fat, feed (ingredients) in ng TEQ/kg)
USA	1957	Feed fat, cow hides, chlorophenols	
USA	1969	Water, chlorophenols	
Japan	1968	Rice oil; PCB-oil	
Taiwan	1979	Rice oil; PCB-oil	
Netherlands	1989	Waste incinerators	Grass; milk 14
USA	1996	Ball clay, feed, chickens, cat fish	Feed 61, cat fish 43 (lw), eggs
Germany	1997	Brazilian citrus pulp, lime, PVC	Pulp 10; milk 4.9; beef 4.3
Belgium	1999	Feed fat, PCB-oil	Feed 2,000; eggs 2,000; chicken meat 3,000; pork
Austria	1999	Kaolinic clay	Clay 1132
Germany, Spain	2000	Choline chloride, sawdust, PCP	Choline chloride 122, feed 0.34
Italy	2001–2004	Mozzarella, waste incineration	Mozzarella (buffalo) 21, sheep milk 30
Germany	2003	Dried bakery waste, waste wood	Bakery waste 12, pork 2.2
Italy	2004	Wood shavings, PCP	Wood shavings 51, eggs 88
Netherlands	2004	Potato peels, kaolinic clay	Peels 44, milk 20
Netherlands	2006	Feed fat, gelatine, HCl	Feed fat 440, feed 8, pork 3
Switzerland	2007	Guar gum	Guar gum 480
Chile	2008	Feed, zinc oxide	Zinc oxide 17,148; feed 14, pork 37
Ireland	2008	Dried bakery waste, PCBs in fuel	Bakery waste 8,500; pork 600, beef, 1,000, pig liver 16,000
Netherlands, Germany	2010	Organic corn, unknown	Corn 2.7; eggs 11
Germany	2010	Industrial fatty acids, chlorophenols	Feed 1.5; eggs, meat

[a]Levels were as reported by the authors and not corrected for different TEF schemes
More details of these incidents can be found from the original references as provided in the source table in Hoogenboom et al. [27]

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Biomonitoring of Dioxins and Furans: Levels and Trends in Humans

Rosana Hernández Weldon and Judy S. LaKind

Abstract Dioxins and furans are ubiquitous, lipophilic, persistent organic chemicals that can be measured in many human tissues and fluids. The most frequently biomonitored human samples are blood and human milk because of the relative ease of sample collection and because these chemicals readily partition into the lipid fraction. In this chapter we will review studies of the general population from around the world that have measured and reported concentrations and toxic equivalency values for dioxin and furans in various human tissues and fluids including milk, blood, umbilical cord blood, adipose tissue, and other tissues that are less frequently analyzed. We will also briefly discuss populations in two areas with excessive dioxin exposure: Vietnam and Seveso, Italy. The majority of the available data indicate that dioxin and furan levels in humans have been declining over the years in the general population as well as in populations with excessive exposure. In addition, there appears to be an age-related difference in levels such that older individuals have higher levels than younger individuals. However, the reader should be aware that many of the available studies are not conducted in a comparable manner and most, including those that are meant to be nationally representative, have small sample sizes. Despite these limitations, biomonitoring of dioxins and furans has been an instrumental tool for establishing baseline levels in humans, discerning trends, informing policies, and assessing the efficacy of policy actions.

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Contents

Abbreviations

2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
2,4-D	2,4-Dichlorophenoxyacetic acid
PCB	Polychlorinated biphenyl
PCDDs	Polychlorinated dibenzo-*p*-dioxins
PCDFs	Polychlorinated dibenzofurans
POPs	Persistent organic pollutants
TCDD	Tetrachlorodibenzo-*p*-dioxin
TEF	Toxic equivalency factor
TEQ	Toxic equivalent
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

1 Introduction

Dioxins are persistent organic chemicals that do not readily degrade in the environment or human/animal tissues and that have been found to adversely affect human health and the environment [1]. Dioxins are primarily unintended by-products of processes including waste incineration, backyard waste burning, and chlorine bleaching of paper and pulp and also form from natural processes such as forest fires. They also occur as contaminants in some pesticides, herbicides, and fungicides [2]. Dioxins are comprised of a large group of chemicals including polychlorinated dibenzo-*p*-dioxins (PCDDs). Additionally, certain compounds that have properties similar to dioxins in terms of toxic mechanisms of action are referred to as "dioxin-like" and include polychlorinated dibenzofurans (PCDFs) and several polychlorinated biphenyls (PCBs). Dioxins and furans are hydrophobic,

lipophilic, and resistant to metabolism in humans. Thus, they have long elimination half-lives in humans, ranging from approximately a few months to a decade, depending on the specific congener (described by the number and location of chlorine atoms on the molecule) and the individual's age and fat content [3]. The toxicity of each congener is typically expressed in terms of toxic equivalency factors (TEFs), a concept that uses the relative potency of a particular dioxin-like compound for producing adverse health effects relative to a reference compound–2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The total toxic equivalent (TEQ) is then used to characterize the toxicity of mixtures of dioxins and/or related dioxin-like compounds in terms of the total 2,3,7,8-TCDD-like activity of the mixture and can be calculated by multiplying the concentration of each compound by its TEF value and then summing the products [4]. Although the method for calculating TEF values has remained relatively unchanged, TEFs of some congeners have been revised over the years as more data have become available about the toxicity of these congeners (see Table 1) [4–6]. These TEF revisions do slightly alter the resulting TEQs; thus, the original research papers may present International TEQs (I-TEQ or NATO/CCMS-TEQ) and World Health Organization TEQs (WHO-TEQ) and may even show the specific year of WHO-TEQ used (1998 or 2005) [7]. The reader should also be aware that some original research articles on dioxin concentrations in humans present the concentrations in several ways. Some may present the actual measured concentration of any particular congener or the sum of congeners without calculating the TEQ, while others may separate or combine TEQs for PCDDs, PCDFs, and dioxin-like PCBs. To make this chapter

Table 1 Summary of TEF values for three of the most commonly used schemes: NATO/CCMS (TEF_I), WHO 1998, and WHO 2005 [4–6]

	TEF_I	WHO_{98}	WHO_{05}
Chlorinated dibenzo-*p*-dioxins			
2,3,7,8-TCDD	1	1	1
1,2,3,7,8-PeCDD	0.5	1	1
1,2,3,4,7,8,-HxCDD	0.1	0.1	0.1
1,2,3,8,7,8-HxCDD	0.1	0.1	0.1
1,2,3,7,8,9_HxCDD	0.1	0.1	0.1
1,2,3,4,8,7,8-HpCDD	0.01	0.01	0.01
OCDD	0.001	0.0001	0.0003
Chlorinated dibenzofurans			
2,3,7,8-TCDF	0.1	0.1	0.1
1,2,3,7,8,-PeCDF	0.05	0.05	0.03
2,3,4,7,8-PeCDF	0.5	0.5	0.3
1,2,3,4,7,8_HxCDF	0.1	0.1	0.1
1,2,3,6,7,8_HxCDF	0.1	0.1	0.1
1,2,3,7,8,9_HxCDF	0.1	0.1	0.1
2,3,4,6,7,8_HxCDF	0.1	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01	0.01
OCDF	0.001	0.0001	0.0003

more accessible, we will present lipid-adjusted dioxin and furan concentrations (excluding dioxin-like PCBs) in standardized units of picograms TEQ per gram lipid (pg TEQ/g lipid) denoting which TEF scheme was used (e.g., $TEQ_I = I{-}TEQ$, $TEQ_{98} = WHO{-}TEQ$ from 1998, and $TEQ_{05} = WHO{-}TEQ$ from 2005), unless otherwise specified. In some cases, the original research papers presented units in part per trillion (ppt) which we converted to pg/g for consistency in this chapter (1 ppt = 1 pg/g).

Humans are primarily exposed to dioxins and furans through the diet. Due to the long elimination half-lives and propensity to partition into fats, these chemicals tend to accumulate in animal tissues and biomagnify through the food chain [3, 4]. Ultimately, meat, fish, and dairy products contribute approximately 90% of the exposure to the general population (US Environmental Protection Agency (June 1994) Estimating Exposure to Dioxin-like Compounds, Vol. II: Properties, Sources, Occurrence and Background Exposures, review draft, unpublished). A wide array of health effects has been associated with dioxin exposure depending on dose and exposure duration [8, 9]. Due to widespread human exposure and concerns regarding the health effects of dioxins and other related chemicals, regulatory agencies around the world – including the US Environmental Protection Agency (USEPA) and more than a hundred countries united under the Stockholm Convention of 2004 – have instituted measures for reducing human exposures to PCDD/Fs. These measures have led to significant decreases in human levels of dioxins over the past 30 years including at least a tenfold decrease for TCDD [10].

The measurement of chemical concentrations in human tissues is termed biomonitoring. Human biomonitoring is a useful tool for characterizing trends and distribution patterns of chemical exposures and identifying new chemical exposures as well as populations which may be vulnerable to exposure [11, 12].

Analytical techniques have been developed to measure dioxins and furans in several human tissues and fluids including blood, adipose tissue, milk, meconium, liver, semen, testes, hair, and others, but dioxins and furans are most frequently measured in blood and milk due to the relative ease of sample collection and the presence of lipids in both fluids [13]. Typically, mass spectrometry techniques are used to quantify concentrations of dioxins in human biological samples [14]. Since the 1960s, some national and international agencies have conducted biomonitoring population surveys of dioxins and other chlorinated chemicals. Examples of these programs are the US National Human Adipose Tissue Survey (NHATS), the US National Health and Nutrition Examination Surveys (NHANES) in which dioxins are measured in blood, the studies of Health Canada in which dioxins have been measured in milk periodically since the 1980s, and the studies conducted by the Canadian Health Measures Survey (CHMS) with serum since 2007. International efforts include the United Nations Environment Programme (UNEP)/World Health Organization (WHO) Global Monitoring Plan which has surveyed mother's milk since 1987 and maternal blood since 2001 [15–17]. These surveys have been used for a variety of purposes, including assessing the effectiveness of global policies instituted to reduce exposures to various chemicals including dioxins.

In this chapter, we provide an overview of levels and trends of dioxins and furans in humans. In Sect. 2, we focus on levels of dioxins and furans in milk, blood, and adipose tissue as these are the matrices for which the preponderance of data exists. We also summarize the limited information on cord blood and adipose tissue, as well as a few other biological matrices. This information in Sect. 2 is not meant to be comprehensive but rather to provide the reader with an understanding of the types of data that are available and how they are used to assess exposure and trends. While our intention is to provide the reader with information that centers principally on populations exposed to background levels of dioxins rather than those influenced by specific local sources, in Sect. 3 we also discuss two communities that had unusually high exposures, one from application of contaminated herbicide and the other resulting from an industrial explosion.

2 Levels and Trends of Dioxins in Human Tissues

In this section, we describe levels, temporal trends, and geographic-specific information on dioxins in human milk (Sect. 2.1), blood (Sect. 2.2), umbilical cord serum (Sect. 2.3), adipose tissue (Sect. 2.4), and other tissues that are less frequently analyzed (Sect. 2.5).

2.1 Dioxins in Human Milk

Hundreds of publications provide data on concentrations of dioxins in human milk. Milk is an ideal matrix for measuring dioxins because of its large fraction of lipids (approximately 4%) [18]. In addition, large sample volumes can be collected noninvasively, and milk serves as a biomarker of exposure for mothers and their developing infants who may be more vulnerable to potential adverse effects.

WHO/UNEP Human Milk Survey: The WHO has been monitoring dioxins in human milk since 1987. In the early years, data were available from only a few regions around the globe. In the past decade, WHO has partnered with the International Programme on Chemical Safety and WHO Global Environmental Monitoring System/Food Contamination Monitoring and Assessment (GEMS/Food) to collect milk samples from women residing in approximately 70 countries. These recent studies include regions with very little historical biomonitoring data such as Africa and parts of Asia [19]. Figure 1 shows the compiled lipid-adjusted $TEQs_{05}$ of PCDD/F measured in the milk of women by participating country and year of sample collection from 2000 to 2012. As shown in Fig. 1, dioxin TEQs in human milk from the vast majority of countries were below 10 pg/g fat, ranging from ~ 1 to 22 pg TEQ_{05}/g fat. India reported the highest levels of PCDD/F in these surveys, while Ethiopia had the lowest levels. The global median of these data was 4.6 pg TEQ_{05}/g fat. These data are particularly valuable both because they serve as a

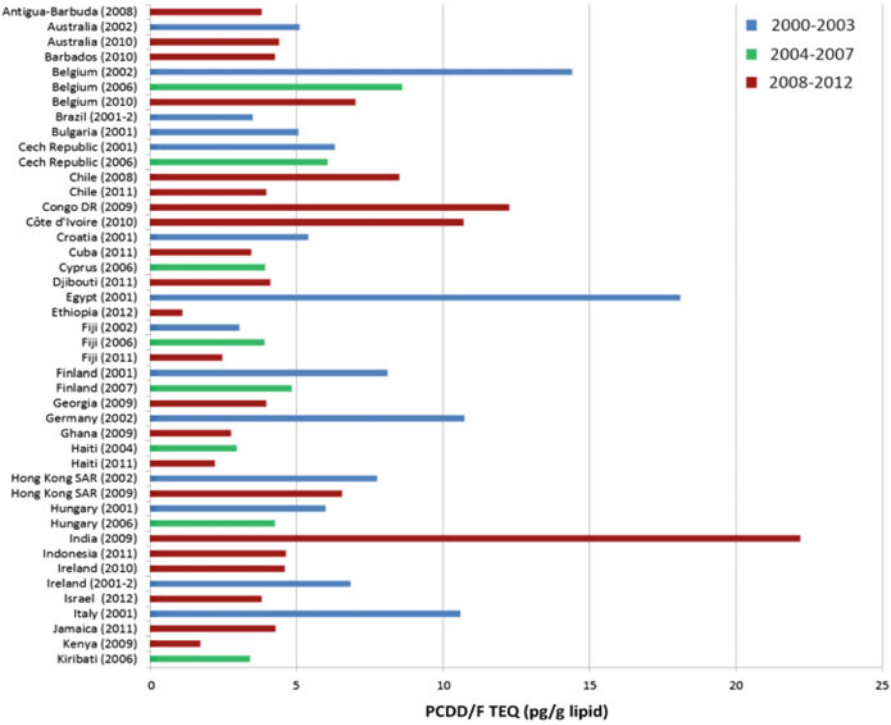

Fig. 1 (continued on next page)

baseline for future surveys and also because samples were collected, processed, and analyzed using the same protocols developed by WHO [20] as guidelines. Thus, data from the different countries and sampling years are more comparable across countries and over time (in contrast, many other studies have been conducted using noncomparable methods making comparison across studies difficult [21]).

Temporal Trends: LaKind et al. reviewed studies on dioxins and human milk published between 1972 and 2005 and examined levels and trends in human milk [21, 22]. While the totality of the global data allowed for an assessment of trends, in the United States there was an insufficient database from which to draw robust conclusions about trends. A 1973 US study reported dioxin $TEQs_{98}$ of 10.8 pg/g lipid in human milk [21, 23], and studies from the 1980s reported dioxin $TEQs_{98}$ ranging between 9.1 and 20.2 pg/g lipid [21, 23–25]. Women from the United States participated in WHO studies in 2002, and under this standardized protocol, median dioxin $TEQs_{98}$ were reported to be 7.2 pg/g lipid [22, 26]. These limited data suggest a decrease in dioxin TEQs in women residing in the United States, but more data are needed to fully elucidate US milk trends.

In countries with more data, decreasing temporal trends are discernible. For example, in Germany, PCDD/F concentrations measured in human milk decreased

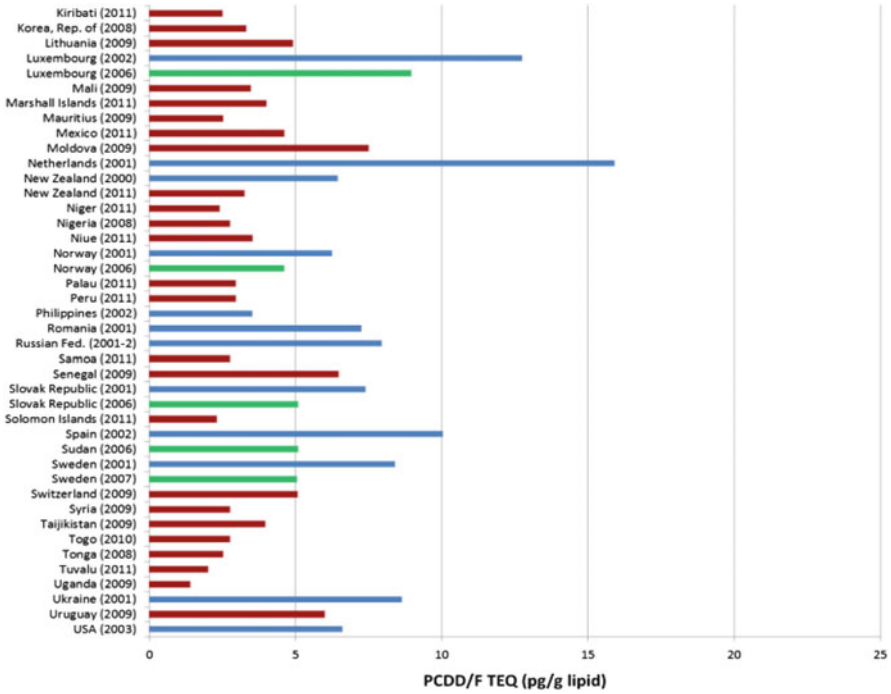

Fig. 1 PCDD/PCDF $TEQs_{05}$ in human milk: WHO/UNEP survey results over the period 2000–2012. Reproduced from Fig. 1 in [20]. The WHO sampling protocol is a guideline, but to promote reliability and comparability, countries were strongly encouraged to follow the protocol as closely as possible. All mothers selected for participation were (a) primiparae, (b) healthy, (c) exclusively breastfeeding one singleton child, and (d) residing in the area for about 5 years. Note that the current WHO sampling protocol requires a minimum of 50 samples per country [20], but earlier studies frequently had fewer samples

by more than half (~35 vs. ~14 pg TEQ_I/g lipid) from 1984 to 1997 [21, 23, 24, 27, 28]. A later German study conducted between 2000 and 2003 reported a median PCDD/F TEQ_I of 11.2 pg/g lipid in human milk suggesting a continuing decline in concentrations but at a lower rate [29]. In the Netherlands TCDD/F $TEQs_{98}$ decreased by about sevenfold from 131.3 pg/g fat in 1985 to 18.3 pg/g fat in 2001 [21–23, 30]. A 2003 Australian biomonitoring study reported a mean of 6 pg TEQ_{98}/g lipid [30, 31] for PCDD/Fs; this value was nearly half of that reported in Australian women in 1993 (11 pg TEQ_{98}/g lipid) showing a decreasing temporal trend as was seen in Europe [31]. Surveys of human milk by Health Canada observed that the PCDD/F TEQ from a composite sample in Canada in 1981–1982 was 25 pg/g lipid [32]; however, by 2005, the mean TEQ_{98} had decreased to 8.3 pg/g lipid [33, 34]. Japan also showed at least a fivefold decrease in PCDD/F TEQ_{98} over two decades (58 pg/g fat in 1980 to 10 pg/g fat in 2000), although there is some variability by region [21, 22]. Overall, however, dioxin concentrations in human milk appear to be decreasing worldwide.

Focusing on Europe, several studies measuring PCDD/F concentrations have been conducted in Sweden, where PCDD/F $TEQs_{98}$ were ~25 pg/g fat in 1987 [21, 24], 8.7 pg/g fat in 2001 [35], and 5 pg TEQ_{05}/g fat in 2007 [19]; the most recent TEQs were ~7 times lower than those reported in Sweden in 1972 (37.8 pg TEQ_{98}/g fat) [23]. In Southern Europe, a study in Athens, Greece, conducted in 2002–2004 exhibited a similar PCDD/F TEQ of 7.3 pg TEQ_{98}/g fat [36, 37].

All of the available published reviews of dioxins in human tissues point to a continued decreasing international trend of dioxin concentrations in human milk [19, 21, 22, 38].

Regional Differences: In 2011, Ulaszewska et al. reviewed available studies on dioxins in human milk published from 2000 to 2009 [38] and reported trends in concentrations of dioxins in milk by continental region. In Asia, dioxin concentrations in human milk varied depending on the country, region within a country, or within the same region. For example, WHO studies of women from Hong Kong reported mean PCDD/F $TEQs_{98}$ of 8.3 pg/g fat in 2002 [39] and 6.6 pg/g fat in 2009 [19]. Human milk PCDD/F TEQs in industrial (Inchon) and urban (Seoul) regions of Korea differed by region within Korea and were both higher than Hong Kong (11.2 pg TEQ_{98}/g fat in Inchon and 24.1 pg TEQ/g fat in Seoul) [40].

Based on the third study period (2001–2003) of the WHO global surveys of human milk [30, 38], the Southern Hemisphere appeared to have lower levels of dioxins than the Northern Hemisphere, but the most recent WHO Human Milk Survey Report suggests that this geographic distinction is not uniformly reflected in the available research. For example, the African continent had the widest variation in PCDD/F TEQs in the most recent WHO milk survey where West and Central African countries such as the Democratic Republic of Congo exhibited a median PCDD/F TEQ_{05} of ~12.2 pg/g lipid, while East African countries such as Ethiopia had only ~1.1 pg/g lipid (Fig. 1) [19]. Further examination of Fig. 1 also shows that PCDD/F TEQs measured in the most northern country (Finland) were similar to those measured in the most southern country (New Zealand) (4.8 vs. 3.3 pg TEQ_{05}/g milk) [19], suggesting that there may not be a simple pattern that varies by hemisphere. Yet, there are regions in the Southern Hemisphere, such as the islands in the Pacific Ocean, that have particularly low PCDD/F $TEQs_{05}$ including Fiji (~2.5 pg/g lipid), Samoa (~2.8 pg/g lipid), and Tonga (~2.5 pg/g lipid) [19].

Summary: From a global view, the available research suggests that concentrations of dioxins measured in human milk have been decreasing over time. This trend is most discernable in countries where studies have been performed repeatedly over decades, such as Germany. The most recent WHO Human Milk Survey expanded the geographical regions that were biomonitored for dioxins in human milk including countries in Africa and several island countries, which provides a better global picture of dioxin exposures to mothers and their infants. Yet, there is some variability in concentrations within continents, countries, and even regions within a country; thus, a single study of a particular region in a country cannot fully capture a country's exposure scenario.

2.2 Dioxins in Blood

Dioxins are commonly measured in blood plasma (the fraction of blood that does not contain red or white blood cells) and, more frequently, in blood serum (the fraction of blood that contains neither blood cells nor clotting factors). A main advantage of measuring dioxins in blood is that exposure can be assessed in all sectors of the population regardless of age or gender, whereas milk can only elucidate exposures in women of reproductive age and their infants. Blood is also relatively easy to collect by venipuncture, although collection in infants and young children is problematic. Since dioxins are lipophilic, concentrations measured in blood are also adjusted by the amount of lipid in the sample; blood serum typically contains less lipid (~0.5%) than milk [41]. Here we provide an overview of key aspects of serum dioxin biomonitoring data from countries in three continents: North America, Australia, and Europe.

United States, *North America*: Dioxins have been measured in serum in the United States as part of the National Health and Nutrition Examination Surveys (NHANES). Dioxins and furans were measured in a random sample of all NHANES participants aged 12 years and older in 1999–2000 ($n = 1271$) and 2003–2004 ($n = 1290$) and in a random sample of NHANES participants aged 20 years and older in 2001–2002 ($n = 1244$) [42–44]. NHANES is meant to be a representative sample of the population, and a subset of samples were chosen randomly from the NHANES population for dioxin analysis. LaKind et al. [45] observed two trends: (i) serum concentrations of dioxins in US populations declined from 1999–2000 to 2003–2004, with median serum PCDD/F concentrations of 13.5 and 11.4 pg TEQ_{05}/g lipid, respectively; (ii) there was an age-dependent association such that the 12–19-year-olds had a median serum PCDD/F concentration of 5.93 pg TEQ_{05}/g lipid as compared to the 60+-year-olds with levels nearly four times higher (22.18 pg TEQ_{05}/g lipid) in 2003–2004. These age-related differences may be related to that fact that older individuals have had a longer duration of exposure to dioxins; thus, the dioxins have had a longer time to accumulate in these individuals. Also, environmental concentrations were higher in the past, so older individuals were likely exposed to higher levels earlier in their life compared to younger individuals [45].

Australia: In 2003, as part of the National Dioxins Program, Australia measured dioxins in 96 blood samples pooled by region, age, and gender from 9,000 individuals aged 0–100 years [46, 47]. Mean serum PCDD/F $TEQs_{98}$ were low (mean = 6.9 pg/g of lipid) compared to most other countries at that time [46, 47]. There were age-related differences in $TEQs_{98}$, with the oldest part of the population having more than twice the concentration as the youngest part of the population (12.9 pg/g lipid in >60 years group versus 3.9 pg/g lipid in the <16 years group). Women over 60 years of age had higher concentrations than men of the same age group (14.6 vs. 11.2 pg/g lipid, respectively), but it is not clear whether this difference is statistically significant.

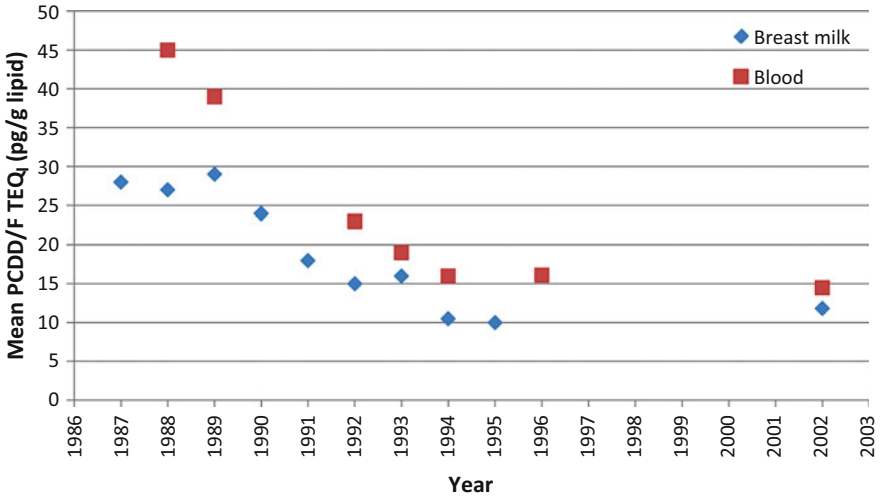

Fig. 2 Trend in PCDD/F TEQs (pg TEQI/g lipid) in human breast milk and blood from 1987–2002, Germany. Data drawn from Papke [41] for years 1987–1996 and Wittsiepe et al. [29] for 2002 data

Germany, *Europe*: Serum dioxin concentrations have been measured in several studies conducted in Germany. In 1998, Papke reviewed the literature on serum concentrations of dioxins from 1988 to 1996 and found a reduction ranging between 50 and 70% as evidenced by a 1996 study of 139 German participants in which the mean TEQ_I was 16.1 pg/g lipid compared to a 1988 study of 10 women whose mean TEQ_I was ~45 pg/g lipid (Fig. 2) [41]. More recent work by Wittsiepe et al. provides additional evidence of the downward trend in concentrations over time with a reported mean TEQ_I for PCDD/F of 14.5 pg/g lipid in 169 breastfeeding women who provided blood samples in 2000–2003 [29].

Summary: Serum data from approximately the same time period (1999–2004) are available to assess trends across the three geographic regions. In general, these data show that Germans have the highest dioxin concentrations in their blood followed by the United States and Australia, although the actual differences between the three concentrations are small. Serum dioxin levels in the United States and Germany show a decline over the past two decades.

2.3 *Dioxins in Umbilical Cord Blood*

Umbilical cord blood can be easily collected at the time of birth and used as a matrix with which to assess fetal exposure to dioxins. During pregnancy, some chemicals, including dioxins, are able to cross the placenta from mother to fetus; thus, concentrations measured in cord blood reflect the amount of exposure a fetus

experienced while *in utero* [14]. Umbilical cord serum has approximately half the lipid content of venous serum (~0.22%) [48]. Several studies indicate that lipid-adjusted cord serum levels of dioxin-like compounds are typically approximately 50% lower than maternal serum levels [48–51].

The preponderance of data on dioxins in cord blood derives from studies conducted in Japan, with a smaller amount of data available from Taiwan and the United States. One 2008 Japanese study reported concentrations of dioxins and furans in cord blood from 49 mother-child pairs and found a mean TEQ_{98} of 7.0 pg/g lipid [51]. This level was lower than the level reported in a 2005 Japanese study of dioxins in cord blood (14 pg TEQ_{98}/g lipid) [50]. Wang et al. reported a mean cord blood PCDD/F TEQ_{98} concentration from 20 participants residing in Taiwan of 4.7 pg/g lipid [48]. A study of five cord blood samples from the United States in 1995–1996 observed similar levels (mean PCDD/F $TEQ_I = 4.7$ pg/g lipid, estimated by the authors from reference [49]).

2.4 Dioxins in Adipose Tissue

Adipose tissue is a major endocrine organ in the body and has many functions including energy homeostasis. Information on concentrations of dioxins in adipose tissue may help elucidate potential relationships between dioxins and obesity-related disease outcomes [52]. In theory, adipose tissue would be ideal for biomonitoring of persistent organic chemicals as the human body is comprised of at least 15–25% adipose tissue, which is mostly lipids, and thus serves as a primary reservoir for these chemicals [52]. However, obtaining adipose tissue samples presents an obvious challenge that limits its use even in small studies. Studies, such as the US Environmental Protection Agency's National Human Adipose Tissue Survey (NHATS), have used data from human adipose tissue from cadavers and surgical patients to estimate average concentrations of dioxins and furans in larger populations [53]. In Table 2, we provide examples of studies reporting on dioxin and furan TEQs measured in adipose tissue. For more comprehensive reviews of dioxins and furans in adipose tissue, see [58, 62].

Studies conducted in Spain (Table 2) suggest decreasing concentrations of dioxins in adipose tissue over time, although as discussed above, small sample sizes and geographic regions limit the generalizability of these studies [59, 60, 62].

2.5 Dioxins in Other Human Tissues

Dioxins have been measured in several other human matrices including semen, testis, amniotic fluid, placenta, hair, saliva, liver, and feces, although to a far lesser extent than milk, blood and adipose tissue. Yet measurements of dioxins in these tissues can yield important information about specialized compartments related to,

Table 2 A sample of published international data on PCDD/F TEQs in adipose tissue

Year	Country	No. donors/ composites	Location (description)	Reference	PCDD/F TEQs (pg/g lipid)	TEF scheme[a]
2006	China	24	Zhejiang Province	[54]	9.2, 7.7	$WHO_{98,\ 05}$
1999	France	16	Paris	[55]	35.6	TEQ_I
~2007	Italy	9	Rome	[56]	11.4, 9.9	$WHO_{98,\ 05}$
1999	Japan	28	Tokyo	[57]	49	WHO_{98}
1998	Spain	15	Catalonia	[58]	31.0	TEQ_I
2003	Spain	20	Granada	[59]	21.5, 19.6	$WHO_{98,\ 05}$
2007	Spain	15	Catalonia	[60]	14.6	WHO_{05}
2004	Turkey	23	Ankara	[61]	9.2	$WHO_{98,}$
1987	United States	865/48	National	[53]	27.9	TEQ_I

[a]TEF schemes are detailed in Table 1

for example, infant exposure (amniotic fluid, placenta, and meconium) [63, 64], or reproduction (semen and testis) [65–67]. In addition, some of these tissues (hair, saliva, and feces) are easy to collect by noninvasive means [64], thus making them attractive for occupational studies. Some important problems associated with using these matrices include: (i) a current lack of ability to interpret the information in terms of exposure and health; (ii) a lack of consistency of the units of the dioxin concentrations across tissues hindering our ability to compare levels across tissues (e.g., dioxin concentrations are typically reported on a dry weight basis in hair rather than lipid-adjusted basis) [64]; and (iii) fundamental differences in the composition of tissue types such that even when the units are consistent across matrices, the meaning of the units may be different (e.g., the type of lipid varies depending on the tissue type – the storage lipids (triglycerides and cholesterol) can be found in adipose tissue and milk, while structural lipids (phospholipids and polar lipids) are found in blood and the liver [68]). These differences are important factors in the interpretation of biomonitoring data. Selected concentration data on dioxins measured in specialized tissues are shown in Table 3. A brief description of some recently published matrix-specific studies follows.

Meconium/Amniotic Fluid: Although studies of dioxins in human meconium and amniotic fluid were not identified, these matrices have been used to measure other persistent chemicals, including dioxin-like PCBs, in humans and dioxin levels in animals [63, 78]. These matrices may provide useful data in future studies of dioxin exposure to infants [64].

Placenta/hair: Researchers have reported that dioxin levels in infant venous serum and cord serum are well correlated with those measured in placenta [48]. It is therefore possible that placenta could be a useful biomarker of fetal exposure. However, it is less clear whether data from hair samples provide an accurate reflection of exposure because of substantial interindividual differences in lipid

Table 3 A sample of PCDD/F TEQs measured in specialized human tissues

Year	Country	No. donors/ composites	Tissue	Reference	PCDD/F TEQs	TEF scheme[a]
1994–1995	Germany	14	Feces	[69]	98 pg/day	TEQ_I
1996	Germany	6	Feces	[70]	863 pg/day	TEQ_I
1994	Japan	6	Hair	[71]	2.1 pg/g dry weight	WHO_{98}
1995	Japan	64 and 68	Hair	[72]	1.0 and 3.2 pg/g dry weight	WHO_{98}
1999–2000	Japan	4	Hair	[73]	25.7 pg/g lipid; 1.1 pg/g dry weight	WHO_{98}
2005	China	5 and 5	Hair	[74]	33.8 and 5.6 pg/g dry weight	WHO_{98}
2007	China	27 and 11	Hair	[75]	36.1 and 2.2 pg/g dry weight	WHO_{98}
1984	Finland	3	Liver	[65]	150 pg/g lipid	TEQ_I
1999	Japan	28	Liver	[57]	57 pg/g lipid	WHO_{98}
2000–2001	Taiwan	20	Placenta	[48]	10.3 pg/g lipid	WHO_{98}
2000–2008	Spain	50	Placenta	[76]	6.9 pg/g lipid	WHO_{05}
2005	China	5 and 5	Placenta	[74]	35.1 and 11.9 pg/g lipid	WHO_{98}
2005	Japan	13	Placenta	[50]	31 pg/g lipid	WHO_{98}
2003	Japan	8	Saliva	[77]	0.07 pg/g lipid[b, c]	WHO_{98}
1991–1992	United States	17/3	Semen	[66]	0.013 ppq wet weight[d]	TEQ_I
1984	Finland	3	Testis	[65]	3 pg/g fresh weight	TEQ_I

[a]TEF schemes are detailed in Table 1
[b]Estimated because data and units are not clear
[c]PCDFs excluded from analysis
[d]Semen data presented as parts per quadrillion on a wet-weight basis due to extremely low lipid levels

content on or in the hair and because the frequency of hair washing may affect measured concentrations [71, 73].

Saliva: Although human saliva is primarily composed of water (~99%) [79], it also contains a small amount of lipid (8–10 mg lipid/100 mL saliva) in which dioxins can be detected. Only one study on dioxins in saliva was identified: Ogawa et al. [77] reported that the concentration of PCDDs in saliva was approximately 10 times lower than that of matched blood samples. While saliva may ultimately prove to be a valuable matrix for measuring dioxins because of the ease of sample collection, additional studies are needed [77].

Feces: Dioxin measurements in feces have been used to attempt to characterize dioxin elimination, equilibrium, and mass balance. For example, Schrey et al. [69] compared intake and excretion of dioxins and found no correlation between current

food intake and excretion; rather, excretion exceeded daily intake for most congeners [69]. The authors proposed that the excess dioxins in feces may be due to the reduction of the body burden and unaccounted sources of dioxins [69]. Rohde et al. [70] found a high correlation ($r > 0.8$) between dioxin concentrations measured in blood and feces, suggesting that fecal excretion of dioxins is regulated by the lipid-based concentrations in the blood.

3 Highly Exposed Populations

In Sect. 2, we focused on levels of dioxins in human populations that were considered to be exposed to background levels of dioxins. There are, however, examples of populations with higher-than-background exposure (e.g., occupational exposure by incinerator workers [80] and accidental poisonings, such as Yusho, Japan, and Yucheng, Taiwan) [81, 82]. In this section, we describe biomonitoring efforts associated with two highly exposed populations: Vietnamese civilians and Vietnam veterans exposed as a result of the US Army's use of dioxin-contaminated herbicides from 1962 to 1971 and residents of Seveso, Italy, exposed in 1976 from an industrial accident [67, 83]. We briefly describe the events surrounding these exposures, the measured concentrations in populations near the time of exposure, and the trends in concentrations since exposure. As the primary dioxin exposure in these populations was TCDD, some of the levels presented are actual measured concentrations of TCDD rather than total TEQ.

Vietnam: Herbicides were sprayed over Vietnam by the US military during the Vietnam War to decrease jungle cover and interrupt food supplies for enemy troops in a military operation known as Operation Ranch Hand. Many of the herbicide mixtures used for defoliation, including Agent Orange – a 50/50 mix of 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) – contained 2,4,5-T that was contaminated with TCDD [84]. Between 2.1 and 4.8 million people lived in regions that experienced direct spraying with these defoliants [84].

The first study of dioxins in the Vietnamese population found concentrations of approximately 77–230 pg TCDD/g lipid in milk samples from women residing in Southern Vietnam [85–87]. Schecter et al. further captured the extent of civilian exposure by measuring dioxins in the milk and blood of women who reported that they had been directly sprayed by Agent Orange or resided in areas that had been sprayed in the South of Vietnam [87]. In these women, initial milk samples taken in 1970 found concentrations as high as 1,832 pg TCDD/g lipid. Follow-up studies nearly 20 years later found concentrations that were below 12 pg TCDD/g lipid, demonstrating a decline of dioxin levels in human milk; caution is required in interpreting these data as sample sizes were small and some concentrations were below the limit of detection at each time point [87]. In 1991–1992, Schecter et al. also measured dioxins in the blood of women who lived in sprayed and unsprayed regions of Vietnam and reported geographical exposure differences;

Vietnamese who resided in South ($n = 433$) or Central ($n = 183$) Vietnam (sprayed areas) had total PCDD/F $TEQs_I$ that were double or triple from those unsprayed Northern ($n = 82$) region (31.3 and 50 vs. 15.3 pg/g lipid, respectively) [87]. Schecter et al. [87] noted several challenges related to sample collection for these studies including military conflicts and the US-imposed economic embargo that was in place from 1978 to 1994. In addition, with the exception of a few of the milk samples from individuals, most of the analyses presented were of pooled samples; thus, it is possible that the pooled levels could be skewed by a few very highly exposed individuals. Nonetheless, these data demonstrate elevated dioxin levels in residents of areas that were sprayed compared to regions that were unsprayed.

American military personnel were also exposed to dioxins during Operation Ranch Hand. A few studies have measured TCDD concentrations in biological matrices, including serum and adipose tissue, of veterans and have generally found higher concentrations of TCDD among veterans who were exposed to herbicides compared to those who were not [86, 88–90]. The largest biomonitoring study of veterans was the Air Force Ranch Hand study: serum dioxin levels were measured in 866 Ranch Hands and 804 controls (controls were other air force personnel involved in missions in Southeast Asia that were unrelated to herbicide spraying) [91]. Serum concentrations among Ranch Hand personnel were higher than the comparison group with median TCDD concentrations in exposed and control groups equal to 12.8 and 4.2 pg TCDD/g lipid, respectively. In addition, there appeared to be a relationship between military rank and exposure: the lowest rank (the enlisted ground crew) had the highest TCDD levels and the highest rank (officers) had the lowest TCDD concentrations (median = 24.0 vs. 7.8 ppt, lipid, for low and high ranks, respectively). Although the Ranch Hand studies are large and extensive, it is worth noting that the study population may be subject to exposure misclassification. Approximately 45% of the Ranch Hands had background serum dioxin levels. It is not clear whether these low levels were due to low initial exposure or study design issues including the 15-year gap between exposure and serum analysis [92].

Seveso: Some of the highest concentrations of TCDD in humans have been measured in residents of Seveso, Italy. On July 10, 1976, a reactor at a chemical plant exploded and released a cloud of chemicals including TCDD, resulting in exposures to thousands of people [93, 94]. Medical examinations of exposed individuals began in 1976, and human blood samples were collected at that time and stored for future analysis [95]. Upon analysis of these stored samples, the highest dioxin concentrations (range = 1,688–56,000; mean = 12,300 pg/g lipid) were found in 2–11-year-old children ($N = 12$) who exhibited an acute symptom of dioxin poisoning – chloracne [93]. Needham et al. further reported a relationship between serum concentration and exposure zone; those who resided in areas assumed to be unaffected by the explosion based on soil samples had the lowest median serum TCDD concentration in 1976 (~15 pg/g lipid). Among 296 residents of all ages who resided in the highest exposure zone (A), the median serum concentration of TCDD was ~450 pg/g lipid [93]. Residents of the zones that

were consecutively less exposed (B and R) had median serum TCDD concentrations of 94 pg/g lipid and 48 pg/g lipid, respectively [93].

Follow-up exposure studies have been conducted on the Seveso cohort in the years since the explosion. Landi et al. [96] reported TCDD concentrations in 62 residents of zones A and B and 59 residents of areas that were considered unexposed from plasma samples collected two decades after the explosion. They found that plasma TCDD concentrations remained elevated in zones A and B (range for zones *A* and *B* = 1.2–89.9 pg/g lipid; median = 73.3 and 12.4 pg/g lipid for zones A and B, respectively) compared to the group considered unexposed (median = 5.5 pg/g lipid) [96]. While TCDD concentrations decreased in the 20 years since the explosion, more importantly, they decreased more than expected based on the 8.2-year half-life estimated for TCDD. This study also demonstrated a gender difference in TCDD levels (higher in women than men in 1996). This difference could not be explained by location within the zone, diet, age, body mass index or smoking.

While TCDD was the chemical that was most studied, some studies looked at other PCDD/F congeners as well in Seveso. One study of the same follow-up cohort [97] and another study focusing on women and children who were exposed in 1976 [98] reported that the elevated total TEQ was largely explained by the elevated TCDD concentrations in the Seveso cohort. There were no other congeners whose concentrations varied by distance from the explosion. In the all-female cohort, the median serum TCDD concentration was 105 ppt lipid in 1976 and 7.3 ppt lipid in 1996 and the 1996 total TEQ concentration was 26.2 pg/g lipid [99].

Summary: The chemical plant explosion in Seveso, Italy, resulted in some of the highest TCDD exposures to human populations in the world. Although the Vietnam studies showed elevated TCDD levels in certain groups who had the most contact with Agent Orange including residents of South or Central Vietnam who were directly sprayed and the ground crew involved in Operation Ranch Hand, these levels were at least ten times lower than some of the residents of Seveso.

4 Discussion

Biomonitoring of various human biological matrices has provided researchers, regulators, and the public with a wealth of information on temporal and geographic trends in exposure to dioxins, as well as information on effects of factors such as age, gender, and diet on exposure. Biomonitoring has also been a valuable tool in following highly exposed populations to better understand changes in levels in those populations over time. In examining the accumulated biomonitoring data on dioxins measured in various tissues, an important and consistent trend is apparent. Generally, regardless of the type of biological matrix sampled, or the region from which samples were derived, dioxin concentrations in humans have been declining over the past few decades. These declines are likely due, at least in part, to policy actions by governmental and international agencies. The 2004 Stockholm

Convention resulted in 178 countries agreeing to reduce exposures to persistent organic pollutants (POPs), including dioxins, by limiting their releases [19, 100]. Evaluation of the Stockholm Convention occurs through regional and global POPs biomonitoring by the World Health Organization, which has provided invaluable baseline and trends data, allowing an evaluation of the efficacy of the efforts to reduce exposures internationally.

Because dioxins partition into lipids, measurements in the lipid fraction of one type of biological matrix should correlate with the level in the lipid fraction of another type of biological matrix. In studies where mothers contributed samples of various biological matrices including maternal blood, placenta, cord blood, and milk, researchers have shown generally high correlation ($r > 80\%$) of concentrations measured, although the levels are not equivalent (e.g., dioxin concentrations tend to be highest in placenta followed by maternal blood, milk, and cord blood) [48–51]. Researchers have proposed regression equations that can be used to relate concentrations measured in some different biological matrices, for example, from serum to milk [48, 101]. The value of this approach is that blood dioxin levels, which are measured in large-scale studies such as NHANES, could be used to estimate levels in human milk, which in turn, could be used to improve infant exposure estimates. It is certainly the case that more research is needed to determine whether some of the special biological matrices mentioned in Table 3 are comparable to the most frequently sampled biological matrices – blood and milk.

Despite the availability of large numbers of studies on dioxins in human tissues, interpretation of the data in these studies must be done with caution. For example, direct comparisons of data across studies may be hampered due to fundamental differences in the populations in each study (e.g., sample size, age, gender, diet). In most of the studies, including those discussed in this chapter, the sample population was not representative of a larger population, and in many cases the sample size was extremely small (e.g., <10). Beyond issues of generalizability and representativeness, differences in aspects of the analytical study components can also render inter-study comparisons difficult, at best. For example, limits of detection can differ among studies, affecting the percent of samples with a detected concentration. Also, it is not always clear which congeners were measured and summed in TEQs. These issues would, in turn, impact study summary data. This problem is exacerbated if the method for assigning a value for measures below the limit of detection differs (e.g., equal to the LOD, ½ the LOD, etc.) [45]. A further problem in comparing data across studies is the lack of harmonization of data reporting, with some studies providing individual-level data and others using pooled data results, as well as differences in the metric used to report data (e.g., ranges, means, medians, lipid-adjusted versus non-lipid adjusted, etc.) [21]. Finally, the method for computing TEQs has evolved, with several revisions given to the TEF scheme [4]. Thus, users of dioxins biomonitoring data need to be aware that older TEQ data are not directly comparable to newer TEQ data. With the consistency of sampling and analysis, the WHO human milk studies serve as a model that should be followed to maximize the comparability of different studies.

Despite these caveats, biomonitoring for dioxins in human tissues has been instrumental in establishing population reference levels, determining the impact of factors such as demographics and diet on these levels, and informing policy decisions. Biomonitoring has also been a critical tool for evaluating the success of policy actions designed to reduce dioxin exposures.

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Contamination Issues in Asian Developing Countries

Tatsuya Kunisue and Shinsuke Tanabe

Abstract This chapter focuses on the contamination, bioaccumulation, and toxicological effects of dioxins and related compounds (DRCs), such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (DL-PCBs), in Asian developing countries, with a particular emphasis on open dumping sites of municipal waste. A comprehensive investigation of soils has suggested clearly that dumping sites (DS) are potential sources of DRCs, whereas the concentrations of DRCs in soils from urban and agricultural areas in Asian developing countries were comparable to or lower than those in general background soils from developed nations. In India, notably higher concentrations of DRCs were detected in human milk from women residing around DS, compared with those from reference sites (RS) and other Asian developing countries, indicating that the residents around DS ingest greater amounts of DRCs, possibly via the intake of contaminated bovine milk and fish. Elevated concentrations of DRCs were also detected in wild animals inhabiting the Indian DS area, such as crow and pig, and the accumulated DRC profiles suggested direct transfer of these contaminants from contaminated soil. Toxic equivalents (TEQs) of DRCs and the liver to adipose concentration ratios of PCDD/Fs in pigs had statistically significant positive correlations with the levels of hepatic cytochrome P450 (CYP) 1A-like protein, suggesting the induction of CYP1A by DRCs and CYP1A-dependent hepatic sequestration of PCDD/Fs. In addition, decreases in plasma-free thyroxine and immunoglobulin G were observed in pigs from the DS. Thus, DS in developing countries are one of the main challenges for further research due to the long-term effects on environmental quality and human/animal health. The continuous formation of DRCs in DS and their elevated residues

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detected in breast milk from residents living around such DS warrant effect studies of these contaminants on their offspring. Comprehensive and long-term monitoring programs are urgently needed with proper capacity building in Asian developing countries, to mitigate DRC emission and their risk on ecosystems and human health.

Keywords Asian developing countries, Bioaccumulation, Cytochrome P450, Dioxins and related compounds, Human exposure, Open dumping sites

Contents

1 Introduction

It is a well-known fact that dioxins and related compounds (DRCs) including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (DL-PCBs) are persistent organic pollutants (POPs). DRCs have been detected in various environmental media and animals including humans because of their persistence in the environment and highly bioaccumulative nature. Especially, higher trophic animals accumulate elevated levels of these contaminants, and consequently their toxic effects have become a social concern [1].

DRCs are unintentionally formed during various combustion processes and are impurities of chlorinated chemicals that were used in large quantities as herbicides and wood preservatives. Combustion is believed to be the major source of PCDDs and PCDFs (PCDD/Fs) to the environment [2]. It has been found that DL-PCBs are also formed in municipal waste incineration [3], while these contaminants are

contained in commercial PCB mixtures [4]. During the past few decades, numerous monitoring surveys on DRC pollution have been conducted mainly in developed nations. In general, emission and exposure levels of DRCs into the environment and for humans have decreased since the 1980s [5]. Despite the fact that DRC contamination has been extensively studied in developed nations, little is known about their behavior, fate, ultimate sources, temporal trends, and animal exposure in developing countries where investigations on DRCs were recently undertaken.

In recent years, public media have voiced concern about open dumping sites (DS) in Asian developing countries where large amounts of municipal solid waste have been dumped. Unfortunately, in most Asian developing countries, open DS areas are located near human habitats; therefore, exposure to various toxic chemicals originating from DS is of serious concern because of the potential effects on human health, wildlife, and environmental quality [1]. Uncontrolled burning of solid waste by waste pickers, generation of methane gas, lack of advanced waste incineration technology, and natural low-temperature burning are major problems in DS at present. These are favorable factors for the formation of DRCs. However, studies on contamination status, animal exposure, and biochemical effects of DRCs are limited in DS.

Our research group has conducted comprehensive investigations of POPs in Asian regions and suggested the presence of DRC sources in Asian developing countries such as India, Cambodia, Vietnam, and the Philippines [6], which have large open dumping sites of municipal waste in the suburbs of major cities. Typically, in DS, a variety of municipal waste are dumped continuously and burnt under low temperature by spontaneous combustion or intentional incineration (Fig. 1). DRCs are formed by this low-temperature combustion, in addition to leaching out of DL-PCBs from dumped electric appliances. Consequently, the surrounding environment may be polluted by these contaminants.

The present chapter discusses the contamination issues and toxicological effects of DRCs in Asian developing countries, with a particular emphasis on open DS from the outcome of comprehensive investigations conducted previously. To date, it is believed that environmental pollution and potential health effects by dioxins are major issues in developed nations. No dioxin problems were known in developing countries other than sporadic incidents such as herbicide agent orange in Vietnam. Here we provide scientific data that DS can be a significant emission source of DRCs that lead to adverse effects on humans and wildlife in developing countries.

Fig. 1 Photos of open dumping sites in Asian developing countries. (**a**) Perungudi dumping site, Chennai city, India. (**b**) Stoeung Meanchey dumping site, Phnom Penh, Cambodia

2 Contamination Status in the Environment: Soil Research

2.1 Residue Levels

To understand the contamination status of DRCs in Asian developing countries, our research group has conducted firstly a comprehensive research of soils [7]. Soil samples were collected from urban and agricultural areas and DS in the Philippines, Vietnam, Cambodia, and India. The DS in each country were located in the suburbs of major cities, Manila (Philippines), Hanoi and Ho Chi Minh (Vietnam),

Phnom Penh (Cambodia), and Chennai (India), and the urban and agricultural areas were more than 30 km away from the DS.

Elevated levels of DRCs were detected in soils from the Asian DS, with the highest concentrations of 200,000 pg/g (dry weight basis) in soils from the Cambodian DS, suggesting the formation and emission of these contaminants in the DS environment. Interestingly, the magnitude of DRC contamination in DS soils was significantly greater than that of urban and agricultural areas. When comparing DRC concentrations in various soil types globally, DS in Asian developing countries showed higher concentrations than general background soils reported in other countries [8, 9] and comparable levels to the DRC-contaminated sites in developed nations [10–12]. On the other hand, DRC concentrations observed in soils from urban and agricultural areas in the Philippines, Vietnam, Cambodia, and India were comparable to or lower than those in general soils from other countries. Furthermore, toxic equivalents (TEQs) of DRCs in some soil samples collected from DS in Asian developing countries, which were estimated based on human/mammal toxic equivalency factors (TEFs) proposed by WHO [13], exceeded the environmental quality standard of 1,000 pg/g TEQs set forth by the Japanese government and US Department of Health [7]. These results suggest clearly that DS are a major source of DRCs in Asian developing countries, while the magnitude of DRC contamination derived from the impurities of agrochemicals and from urban activities was relatively small by comparison. Though reaction mechanisms for DRC formation are believed to be complex, the combustion of chlorinated waste is the major source of PCDD/Fs to the global environment [14]. A previous study evaluated the contribution to dioxin formation from combustion of some polymer materials such as polyethylene (PE), polystyrene (PS), and polyvinyl chloride (PVC) and showed that PVC contributed significantly to the formation of PCDD/Fs and DL-PCBs [15]. Common applications of plastics in daily use products and industries together with the lack of proper management of waste materials in developing countries have led to significant disposal of chlorinated waste-containing products such as PVC, chloromethane, and chlorophenols in open DS every day. Accordingly, we suggest the possibility of the considerable formation of DRCs in these DS.

2.2 Homologue Profiles

To further understand the role of DS as a source of DRCs, homologue profiles of PCDD/Fs were examined in DS soils. Their PCDD/F homologue profiles were then compared with typical profiles of samples representing environmental sources, which were the emission from a typical municipal waste incinerator in the United States [14] and an average of 12 different combustion sources [16] (Fig. 2). In general, the homologue profiles of samples representing environmental sources are characterized by the predominance of lower chlorinated dibenzofurans and an increasing proportion from tetra- to hexa-chlorinated dibenzo-*p*-dioxins (T_4-H_6CDDs). Interestingly, homologue profiles of the DS soils from the Philippines,

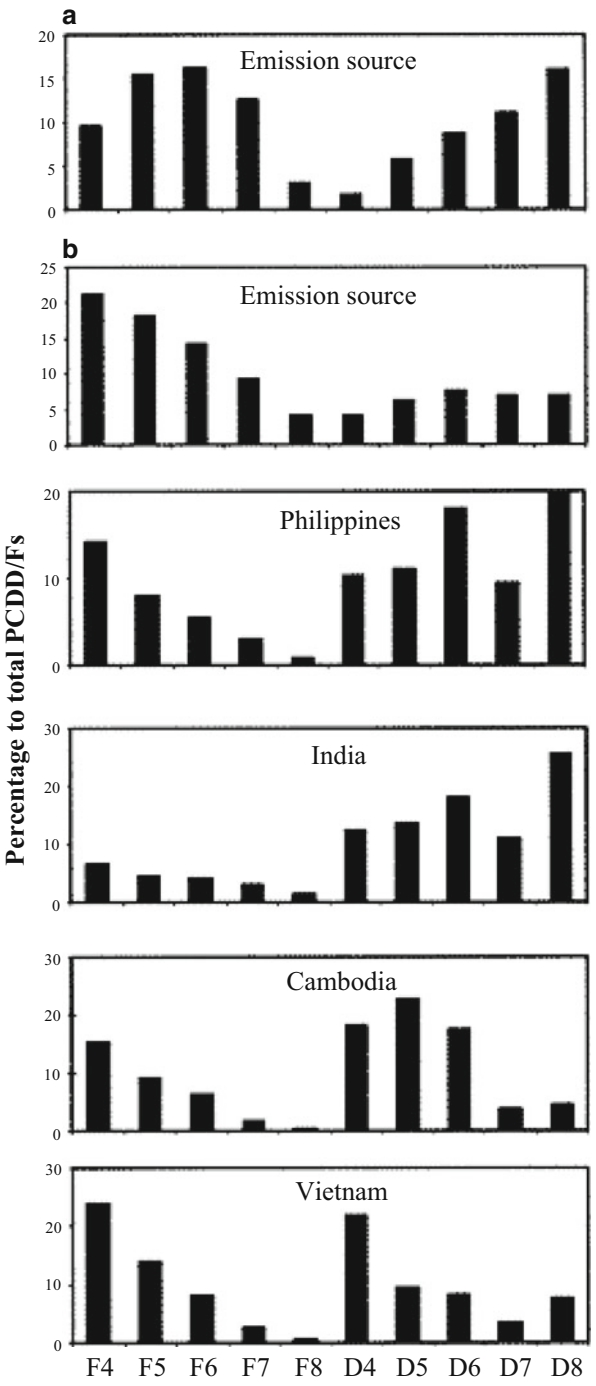

Fig. 2 Homologue profiles of PCDD/Fs in soils from dumping sites in Asian developing countries in comparison with the profile of samples representing emission sources (municipal waste incinerators). Vertical bars represent the percentage of each homologue to total PCDD/F concentrations. F and D refer to dibenzofurans and dibenzo-*p*-dioxins, respectively, and numbers indicate the degree of chlorination. Data for emission source samples were cited from Brzuzy and Hites [14], (**a**) a typical emission of a municipal waste incinerator, and Baker and Hites [16], (**b**) an average of 12 different combustion sources

Vietnam, Cambodia, and India reflected a pattern of emission sources (Fig. 2). PCDD/F profiles of DS soils from the Philippines and Cambodia were similar to those of emission sources, implying recent formation of these contaminants in each DS. On the other hand, the typical pattern of environmental sink samples, which were soils collected from various locations over the world, contains octachlorinated dibenzo-*p*-dioxin (O_8CDD) as a predominant congener [16]. PCDD/F profiles observed in the urban and agricultural soils from Asian developing countries were similar to those of typical environmental sinks [7].

As for DL-PCB congener patterns, non-*ortho* congener CB-126 contributed predominantly to total TEQs in most of the soil samples surveyed in Asian developing countries. The formation of DL-PCBs has been hypothesized through three alternative processes including the release from commercial PCB mixtures, emission from combustion, and, to a lesser extent, photolysis of higher chlorinated PCBs [17]. A study in the United Kingdom reported that TEQ input of DL-PCBs from Aroclor formulations into the environment was mainly contributed by CB-77, CB-105, CB-118, CB-156, and to a lesser extent CB-126 [18]. Combustion source emissions were dominated by non-*ortho* DL-PCBs, in which CB-126 contributed predominantly to total TEQs [17]. In addition, it should be noted that CB-126 can be formed during the domestic burning process [19]. Our result suggests that uncontrolled burning of solid wastes in Asian DS could be a source of DL-PCBs.

2.3 Flux and Load of DRCs to DS

Soil is a useful environmental matrix to estimate the deposition of PCDD/Fs on a global scale [9, 14, 20]. Using the same approach that was reported in the previous studies [9, 14, 20], we estimated the flux of PCDD/Fs to the soils and their load to the DS areas in Asian developing countries. Flux to soils can be calculated by the following equation:

$$F = CM/(St),$$

where F is depositional flux to soils (ng m^{-2} $year^{-1}$), C is the concentration in soils, M is the mass of soils collected (g), S is the surface area of soil sample (m^2), and t is the accumulation time of PCDD/Fs in the soil compartment (year). For soils in open DS, t values were calculated on the basis of the time when DS began to be used and the time when soil samples were collected [7]. Accordingly, we set t values for soils in DS in the Philippines; Cambodia; India; Hanoi, Vietnam; and Ho chi minh, Vietnam, at 7, 21, 15, 3, and 11 years, respectively. The loading rate (R) of PCDD/Fs to a DS (considered as the annual amount of PCDD/Fs received by surface area of the DS, mg TEQs/year) can be calculated by multiplying flux value to surface area (A) of the DS:

Table 1 Estimated flux of PCDD/Fs to dumping site soils and estimated loadings of PCDD/Fs to dumping site areas in Asian countries

Country	Flux (ng m^{-2} $year^{-1}$)		Dumping site area (m^2)	Load	
	Mean	Range		mg/year	mg TEQ/year
Philippines	17,000	13000–21,000	230,000	3,900	35
Cambodia	2,900	31–19,000	30,000	87	1.1
India	990	290–4,500	1,400,000	1,400	8.8
Vietnam–Hanoi	4,100	83–34,000	50,000	210	3.2
Vietnam–Ho Chi Minh	67	3.8–160	300,000	20	0.12

$$R = FA.$$

Estimated fluxes of PCDD/Fs to soils in DS in the Philippines, Cambodia, India, and Vietnam are given in Table 1. It is interesting to note that fluxes to DS soils from the Philippines and Cambodia were greater than those from other locations in the world reported previously [9]. This result indicates that DS are potential sources of PCDD/Fs; the elevated fluxes observed in these DS could be attributed to uncontrolled combustion processes. The load of PCDD/Fs to the DS indicates that DS in the Philippines and India with a large area of approximately 23 and 140 ha could receive the highest annual amount of 3,900 and 1,400 mg/year PCDD/Fs (35 and 8.8 mg TEQs/year), respectively (Table 1). The DS in Ho chi minh, Vietnam, had the lowest loading rate due to the less contamination of PCDD/Fs in soils. For comparison, total annual fluxes to the Kanto region in Japan, one of the polluted areas in the world, were estimated to range from 50 to 900 g TEQ with a total area of 32,000 km^2 (approximately 3 million ha) [21]. The area of DS in India is 140 ha, which is 21,000 times smaller than that of the Kanto region, and this area was estimated to receive 8.8 mg TEQs/year. These estimates suggest that DS in India and the Philippines may be significant reservoirs for PCDD/Fs [7].

3 Human Exposure: Human Milk Research

3.1 Contamination Status

To evaluate the status of human exposure to DRCs, we have analyzed human breast milk from Asian countries [22]. Mean lipid-normalized concentrations of TEQs of DRCs, which were estimated based on human/mammal TEFs proposed by WHO [13], in human breast milk collected from general public in India, Cambodia, Vietnam, the Philippines, Malaysia, China, and Japan [23–26] are illustrated in Fig. 3. Relatively high concentrations of TEQs were found in human milk from Japan where a large amount of DRCs have been released into the environment in the

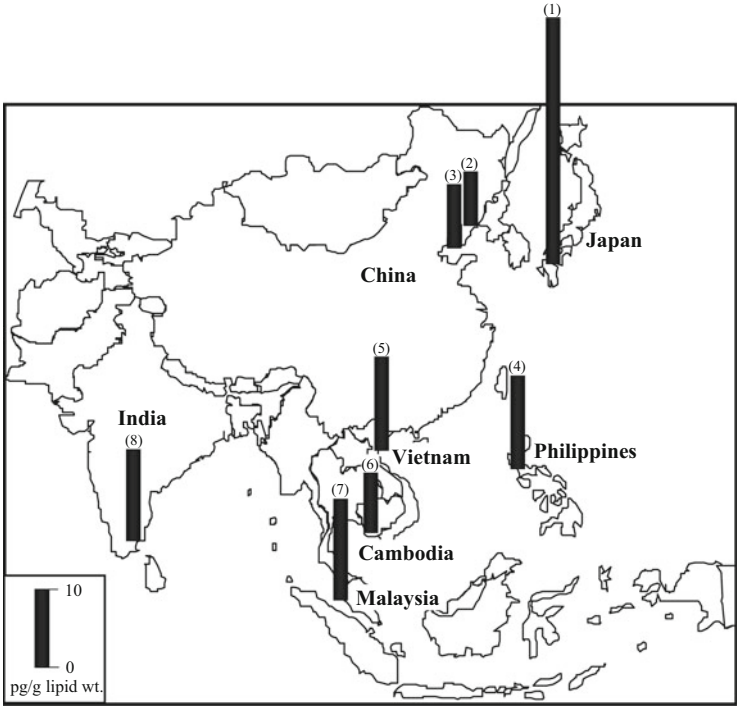

Fig. 3 TEQs of DRCs (PCDDs, PCDFs, and DL-PCBs) in human breast milk collected from general public in Asian countries. (*1*) Fukuoka, Japan (Kunisue et al. [23]), (*2*) Shenyang, China (Kunisue et al. [24]), (*3*) Dalian, China (Kunisue et al. [24]), (*4*) Quezon, Philippines (Kunisue et al. [25]), (*5*) Hanoi, Vietnam (Kunisue et al. [25]), (*6*) Phnom Penh, Cambodia (Kunisue et al. [25]), (*7*) Penang, Malaysia (Sudaryanto et al. [26]), (*8*) Palaverkadu, India (Kunisue et al. [25])

past. Our studies also demonstrated elevated DRC concentrations in wildlife inhabiting Japan [27, 28]. From the outcome of our soil survey described above, we presumed that the residents living around DS may be exposed to DRCs, because most of them earn their livelihood by doing DS-dependent labor. It is expected that in utero and lactational exposure to DRCs may adversely affect the brain development and immune systems of infants and children [29–32]. So, we attempted to elucidate the contamination status of DRCs in human breast milk collected from the residents around DS in India, Cambodia, and Vietnam and compared with those in general public from reference sites (RS) [25].

In India, the concentrations of DRCs in human breast milk from the DS were significantly higher than those from the RS and the other two countries, while levels of these contaminants in human breast milk from Cambodia and Vietnam were not significantly different between the DS and RS (Fig. 4). This result indicates that significant pollution sources of DRCs are present in/around the DS of India, and the surrounding residents may be exposed to relatively high levels of these

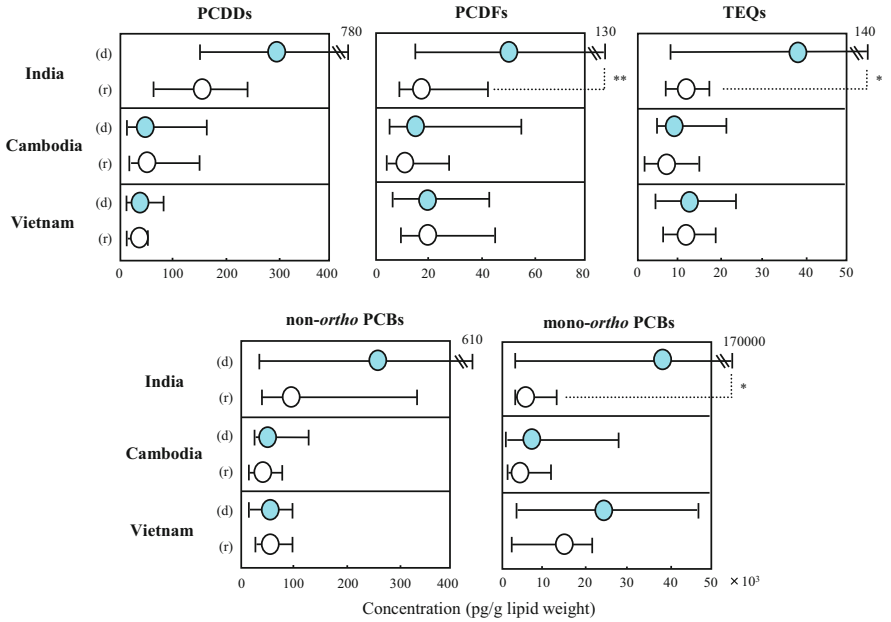

Fig. 4 Comparison of DRC concentrations in human breast milk from dumping (d) and reference (r) sites. The circles and bars represent mean and range values, respectively. $*p < 0.05$, $**p < 0.01$. Data were cited from Kunisue et al. [25]

contaminants. To understand the magnitude of contamination in human breast milk from the Indian DS, TEQ levels were compared with the values for human breast milk from general public of other countries since 1990. The TEQ levels in human breast milk from the Indian DS were comparable to or higher than those from developed countries [23, 33–38], suggesting that the DS residents have been exposed to comparable levels of DRCs with the general public in developed countries. On the other hand, the TEQs in human breast milk from Cambodia and Vietnam were lower than those from developed countries and comparable to those from other developing countries [24, 26, 37, 39]. In this international comparison, however, there are some uncertainties such as age and parity of the mother, sampling period, sample number, and accuracy of the analytical techniques involved. In addition, very little data are available on mono-*ortho* DL-PCBs in the literature. Because of such uncertainties, it was difficult to draw any firm conclusion from the above comparison. However, the observation that TEQ levels in human breast milk from the DS of India were comparable to or higher than TEQ values, which were estimated from PCDD/F and DL-PCB concentrations, from some developed countries including Japan is noteworthy. In developed countries, concentrations of DRCs in human breast milk have recently decreased [40], because of the installation of highly efficient incinerators and strict regulations on the production and usage of various chemicals. On the other hand, in Asian developing countries, it can be anticipated that the residue levels of DRCs in

human breast milk may increase in the future, because the release of these contaminants is poorly controlled currently.

3.2 Variation Associated with Parity and Age

Concentrations of DRCs in human breast milk vary by various factors such as the age, parity, and breast-feeding period of the mother [40, 41]. In the case of primiparae, it is observed that DRC concentrations in human breast milk were positively correlated with the age of mothers [23]. However, our study on Asian developing countries showed no significant correlations between DRC concentrations and primiparae age [25]. Although it cannot be clearly explained why no significant correlation was observed in Asian developing countries, a narrow range of age and recent exposure of DRCs may be possible reasons. Most women in Asian developing countries have many children in their life with the first infant often born by the mother at a young age.

In developing countries, it can be anticipated that the parity of mother is one of the focal factors influencing concentrations of DRCs in human breast milk. Therefore, we examined the relationship between the number of deliveries by the mothers and TEQs in human breast milk from women in Asian developing countries. TEQ levels in human breast milk from the DS of India tended to decrease with increase in the number of deliveries. One of the primipara donors had an exceptionally high TEQ level (140 pg/g lipid wt.). These results suggest that mothers who have been exposed to relatively high levels of DRCs may transfer higher amounts of these contaminants to the first infant than to the infants born afterward through breast-feeding, and hence the firstborn children might be at higher risk by DRCs. In developed countries, DRC concentrations in human breast milk from primiparae were also higher than those from multiparae [23, 41].

3.3 Risk Assessment for Infant

The presence of DRCs in human breast milk is of great concern, because these lipophilic chemicals are readily transferred and absorbed to infants. It is reported that one- to three-month-old infants absorb above 90% of most DRC congeners containing in their mothers' milk [42–44]. To understand the magnitude of exposure to DRCs by infants, we estimated daily intake (DI) from the concentrations of these contaminants in human breast milk observed in Asian developing countries, based on the assumption that an infant ingests 700 ml milk per day and the weight of an infant is 5 kg, and compared to the guideline standard proposed by the WHO [45]. As expected, relatively higher DIs of TEQs were observed in infants residing around DS in India compared with those from other countries, and DIs in all cases exceeded 1–4 pg TEQs/kg/day, the tolerable daily intake (TDI) (Fig. 5). DRCs

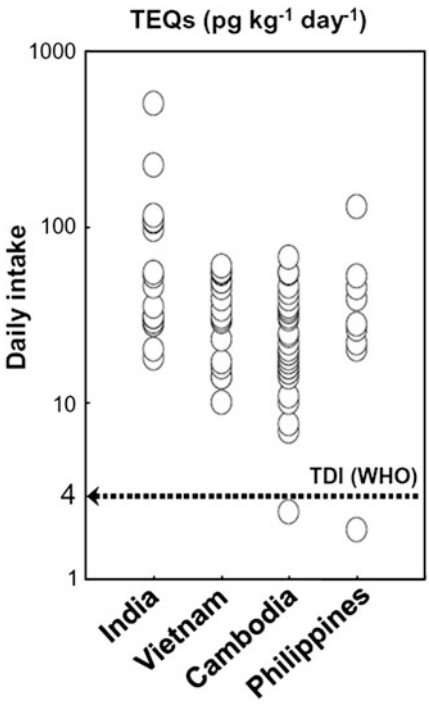

Fig. 5 Estimated daily intake (DI) of DRCs (TEQs) by infants in Asian developing countries. DI was estimated using TEQ data in human breast milk reported by Kunisue et al. [25]. TDI: Tolerable Daily Intake [45]

induce various toxic effects, e.g., cancer, in animal bodies [46]. These observations imply that abundance of DRCs in human breast milk may adversely affect development and reproductive systems of Asian children. However, it is difficult to draw any firm conclusions from Fig. 5 whether or not adverse effects by DRCs have already occurred in Asian infants, because TDIs used here are estimated on the basis of life-span exposure. Not only TDIs from life span but also TDIs of DRCs estimated from breast-feeding period are needed.

4 Potential Sources for Dumping Site Residents

4.1 Bovine Milk

Although greater contamination of DRCs was observed in DS soils compared to urban and agricultural soils in Asian developing countries [7], the DRC concentrations in human breast milk collected from the DS residents in Cambodia and Vietnam were not significantly higher than those from RS. As described earlier, however, residue levels of DRCs in Indian samples from the DS were notably higher (Fig. 4). These observations imply that the residents around the DS in Cambodia and Vietnam have not been greatly exposed to DRCs originating from

the DS. For humans, food intake, especially meat and dairy products, accounts for 98.8% of exposure to DRCs, and consumption of water, ingestion of soil, and inhalation of air are not major sources [47]. In addition, residue levels and composition of DRCs in human tissues generally reflect those in foods ingested [48–51]. In India, buffalo and cows reared near the DS feed mainly on dumped leftovers (Fig. 6). The residents around the DS constantly drink the milk collected from these bovines (Fig. 6). On the other hand, in Cambodia and Vietnam, livestock such as buffalo and cows are not reared around the DS. To elucidate whether or not bovine milk is a potential source of DRCs for the residents around the DS in India, residue levels of these contaminants in buffalo' and cows' milk collected were investigated and compared with those in bovine milk collected from RS [25].

DRCs were detected in all of the bovine milk samples analyzed, revealing that bovines in India have been exposed to these contaminants. Concentrations of DRCs in bovine milk collected from the DS were significantly higher than those from the RS (Fig. 7). This result indicates that buffalo and cows feeding in the Indian DS consume greater amounts of DRCs through contaminated soils and/or garbage and that daily intake of these bovine milk by the residents around the DS is one of the possible reasons why elevated TEQ levels were observed in human breast milk collected from the DS. Interestingly, compositions of PCDD/F congeners in bovine milk showed different patterns depending on the area of collection. In bovine milk collected from the DS, lower chlorinated congeners such as 2,3,7,8-T_4CDD, 1,2,3,7,8-P_5CDD, and 2,3,4,7,8-P_5CDF predominated, while the residue levels of 1,2,3,4,6,7,8-H_7CDD and O_8CDD were relatively high in those from the RS (Fig. 8). As described in our soil study, concentrations of T_4-, P_5-, and H_6-CDD/Fs in soils from the DS in India were higher than those from urban and agricultural areas [7]. These observations indicate that T_4-, P_5-, and H_6-CDD/Fs are formed via combustion of municipal waste and that buffalo and cows feeding in and around the Indian DS accumulate greater amounts of these compounds through contaminated soils, leftovers, and/or pastures. In soils collected from Indian DS, however, 1,2,3,4,6,7,8-H_7CDD and O_8CDD were predominant among all the 2,3,7,8-substituted congeners [7]. A previous study reported that the average percent contribution of high-chlorinated DD/Fs in pastures effected through soil particle adhesion was higher than low-chlorinated DD/Fs, and during summer, the period of high atmospheric temperature, the uptake of PCDD/Fs by pasture from vapor phase increased with the increasing degree of chlorination (increasing K_{OA}) [52]. Additionally, another study showed that PCDD/F contamination in cow milk reflected not only the intake from pastures but also ingestion through contaminated soils [53]. These findings suggest that the intake of high-chlorinated DD/DFs such as 1,2,3,4,6,7,8-H_7CDD and O_8CDD by buffalo and cows in and around the Indian DS is greater than that of low-chlorinated DD/DFs. In bovine milk from the DS, however, higher concentrations of low-chlorinated DD/DFs such as T_4-, P_5-, and H_6-CDD/Fs were observed, indicating that buffalo and cows in and around the Indian DS preferentially transfer more amounts of low-chlorinated DD/Fs to their milk. Fries et al. [54, 55] investigated a mass balance of PCDD/Fs in cows following administration of pentachlorophenol-treated wood, and they reported

Fig. 6 Photos of buffalo and cows rearing in/around Perungudi dumping site, Chennai city, India

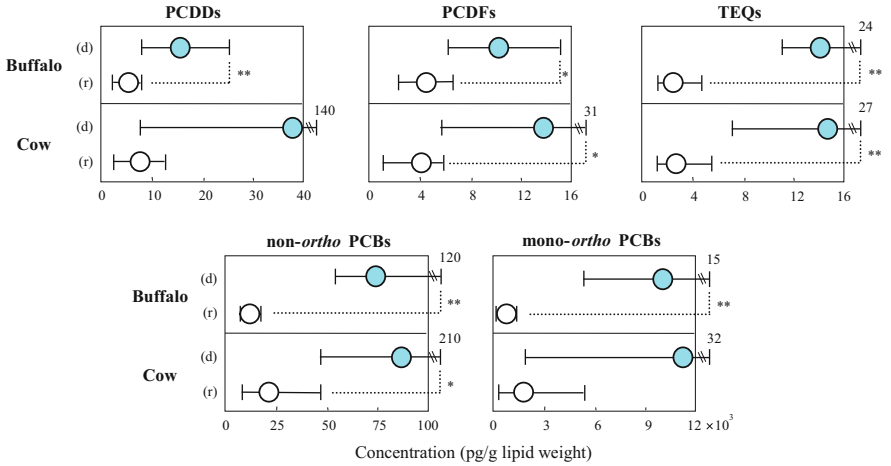

Fig. 7 Comparison of DRC concentrations in bovine milk from dumping (d) and reference (r) sites in India. The circles and bars represent mean and range values, respectively. $*p < 0.05$, $**p < 0.01$. Data were cited from Kunisue et al. [25]

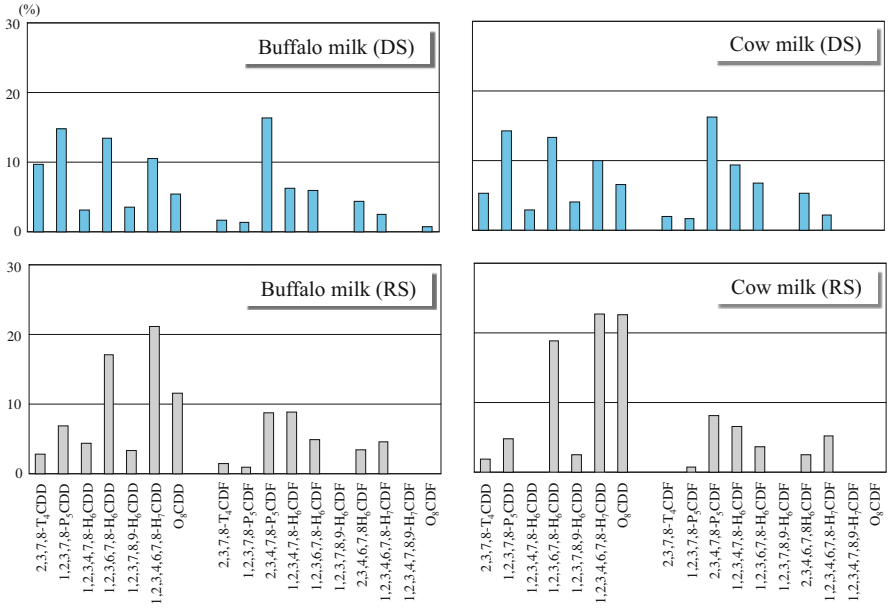

Fig. 8 Compositions of PCDD/Fs in bovine milk collected from the dumping and reference sites in India. DS and RS in the parentheses represent dumping site and reference site, respectively. Data were cited from Kunisue et al. [25]

that transfer to milk and storage in body fat increased with decreasing degree of chlorination, while excretion in feces increased with increasing degree of chlorination. Thus, bovines from the Indian DS transfer considerable amounts of low-chlorinated DD/Fs to their milk. Residents who constantly drink bovine milk are at high risk because of their high TEF values. In India, dietary consumption of dairy products is generally higher than other countries, and average consumption of milk in India by a person per day rose from 135 g in 1980 to 176 g in 1990 [56]. Assuming that an adult weighing 60 kg drinks 176 g of the buffalo or cow milk investigated in India per day, the estimated daily intake of TEQs from bovine milk from the DS was above 1 pg TEQs/kg/day, and only one buffalo milk sample had a value that exceeded the TDI proposed by the WHO [45] (Fig. 9). Even though the values are within TDI, the residents around the DS in India are exposed to considerably high levels of DRCs and hence may be at greater risk of exposure to these contaminants via bovine milk.

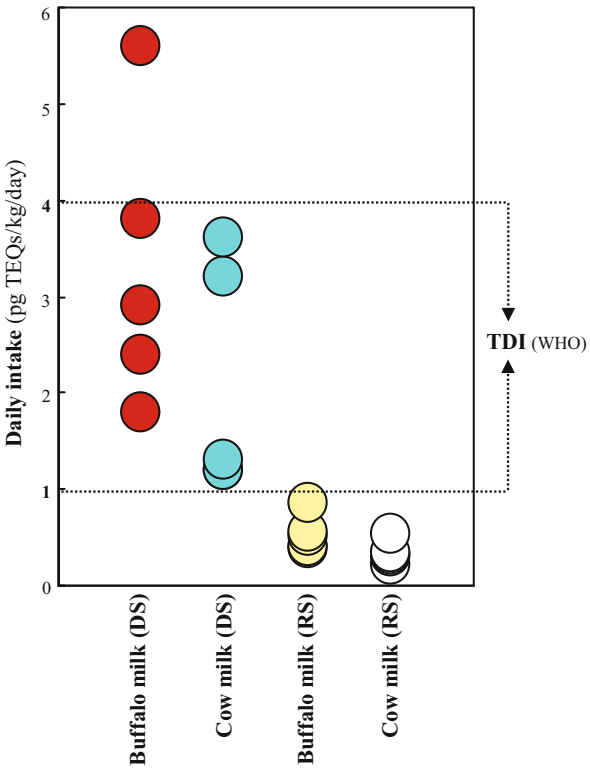

Fig. 9 Estimated daily intake of TEQs by adults through bovine milk collected from the dumping and reference sites in India. DS and RS in the parentheses represent dumping site and reference site, respectively. Daily intake was estimated using TEQ data in bovine milk reported by Kunisue et al. [25] and based on the assumption that an adult (60 kg) ingests 176 g of bovine milk per day (John et al. [56])

4.2 Fish

Recently, we have detected elevated concentrations of DRCs, especially DL-PCBs, in human breast milk collected from residents around a DS in Kolkata, India, which is located in the northeastern region and is the second largest city in India [57]. The TEQ levels were higher than those in human breast milk from the DS in Chennai described earlier [25] and recent levels in Japanese milk [23] (Fig. 10). These observations indicate that the magnitude of pollution by DRCs in Indian DS could be different domestically, and the residents around such DS ingest greater amounts of DRCs compared with general public in developed countries. Unlike the DS in Chennai, livestock animals such as buffalo and cows were not reared around the DS in Kolkata. However, there is a pond adjacent to the DS in Kolkata, and the interview with the DS residents showed that they consume fish collected from the pond. When the relationships between DRC concentrations in human breast milk and frequency of food consumption by the DS residents were examined, the DRC concentrations significantly increased with the frequency of fish consumption, but not with those of meat and dairy products [57]. Extremely high concentrations of DRCs (mean: 500 pg TEQs/g lipid wt.) were detected in fish collected from the pond adjacent to the DS, compared with those (31 pg TEQs/g lipid wt.) in fish collected from a RS pond. These results clearly suggest that fish consumption is a major source of DRCs for the DS residents in Kolkata. Furthermore, assuming that an adult weighing 60 kg eats 30 g of fish investigated in Kolkata per day, estimated daily intake of TEQs (4.6–16 pg TEQs/kg/day) from DRC concentrations in fish samples collected from the pond adjacent to the DS exceeded the WHO-TDI [45].

Considering the above observations, it is likely that not only residents are affected by DRCs, but also many other animals inhabiting the DS areas may be exposed to considerably high levels of DRCs derived from the DS and may suffer adverse effects.

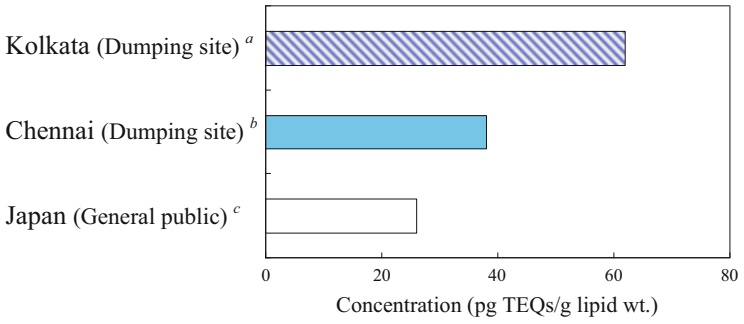

Fig. 10 Comparison of TEQ levels in human breast milk collected from the residents around dumping sites in Kolkata and Chennai and from the general public in Japan. [a]Someya et al. [57], [b]Kunisue et al. [25], [c]Kunisue et al. [23]

5 Animal Exposure and Toxicological Impacts

In the DS of Chennai in India, wild crows (house crow [*Corvus splendens*] and jungle crow [*Corvus macrorhynchos*]) and pigs (*Sus scrofa*) feed on the raw garbage with contaminated soils (Fig. 11). Because biomagnification of DRCs through the contaminated garbage and further adverse effects on these animals are speculated, our group clarified the contamination levels and accumulation features of DRCs and assessed the biochemical effects in crows and pigs inhabiting the DS [58, 59].

Fig. 11 Photos of crows and pigs in Perungudi dumping site, Chennai city, India

5.1 Crow

5.1.1 Accumulation Patterns

DRCs in pectoral muscle samples of crows collected from the DS and a RS in Chennai were analyzed. As with humans and bovine described earlier, the concentrations of DRCs detected in the DS crows were significantly higher than those from the RS [58]. Another study showed that DRC concentrations in general population of various animals from India were lower than those reported in Japan [60]. However, the magnitude of DRC contamination in crows from the Indian DS was significantly greater than those from Japan [58], suggesting that crows in the DS have been exposed to these contaminants derived from burning wastes by their DS feeding activity. When the compositions of DRCs were examined, the profiles observed in DS crows were similar to those in soils collected from the DS [7], but not for those in crows from the RS. This result indicates that crows inhabiting the DS ingest contaminated soil together with raw garbage. A further scale-dependent analysis, principal component analysis (PCA), supported that the DRC profiles in crows from the DS were influenced by DRC congeners present in the DS soils [58].

5.1.2 Bioconcentration

To verify whether or not the DRC congeners in crows from the DS were directly affected by on-site contamination, bioconcentration factors (BCFs) of PCDD/F congeners were estimated from concentrations in crows and soils from the DS, and compared with the theoretical BCF values, which were calculated from water-particle and lipid-water partitioning coefficients. BCFs of individual congeners in crows from the DS were calculated as the ratios of concentrations in crow muscles (C_{lipid} on lipid-weight basis) to the concentration in soils (C_{particle} on dry weight basis); $\text{BCF}_{\text{measured}} = C_{\text{muscle}}/C_{\text{soil}}$. In addition to BCFs based on the congener concentrations in muscle and soil, theoretical BCFs ($\text{BCF}_{\text{theoretical}}$) were calculated assuming that transfer of congeners contributed to the body of crow from the DS was dependent upon their partitioning between soil particle and lipid in tissue. The partition of individual congeners between particle and lipid was estimated using the following formula:

$$\text{BCF}_{\text{theoretical}} = \frac{C_{\text{muscle}}}{C_{\text{soil}}} = \frac{C_{\text{lipid}}}{C_{\text{particle}}} = \frac{C_{\text{water}}}{C_{\text{particle}}} \times \frac{C_{\text{lipid}}}{C_{\text{water}}} = \frac{1}{K_{\text{pw}}} \times K_{\text{bw}},$$

where particle-water partition coefficient (K_{pw}) and biotic lipid-water partition coefficient (K_{bw}) were referred from Govers and Krop [61]. Regression analyses revealed that the averages of log $\text{BCF}_{\text{measured}}$ calculated in the DS crows were positively correlated with log $\text{BCF}_{\text{theoretical}}$ (Fig. 12). This result elucidated that

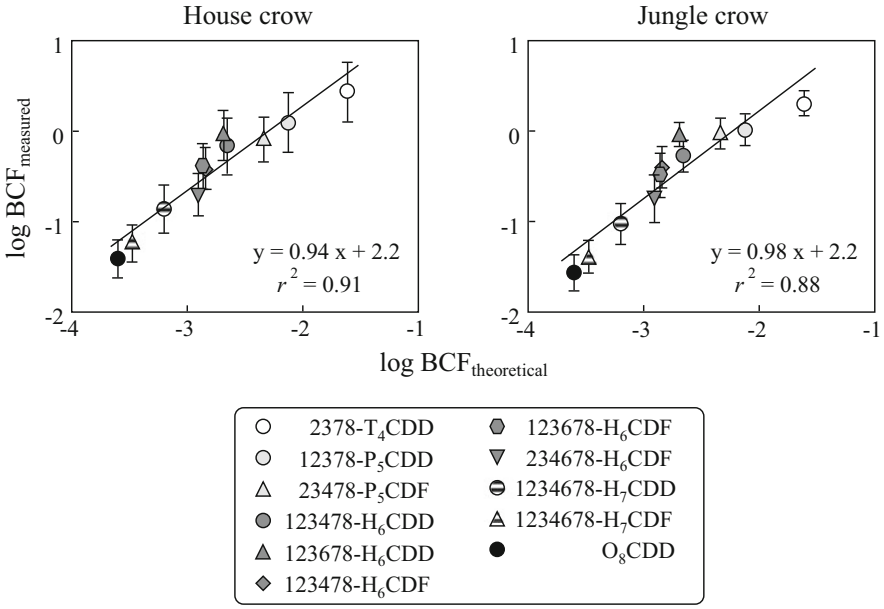

Fig. 12 Relationships between log $BCF_{measured}$ and log $BCF_{theoretical}$ for PCDD/Fs in crows from the Indian dumping site. Each plot and bar means average and standard deviation, respectively. Data were cited from Watanabe et al. [58]

PCDD/F congener profiles in the muscle of crows from the DS were mostly determined by the soil particle-lipid partitioning, based on the physicochemical characteristics of each congener. However, the $BCF_{measured}$ was approximately 10^2-fold higher than the $BCF_{theoretical}$. Some factors including intake pathway and diet composition might have influenced the distribution of PCDD/Fs in crows. The K_{bw} used for estimating $BCF_{theoretical}$ was calculated from the experimental data on fish exposed to PCDD/Fs dissolved in water [61]. In fish, the major entry route of xenobiotics dissolved in water is a direct pathway across the blood-water interface at the gills, and uptake of xenobiotics in the diet can be ignored when estimating internal concentration of xenobiotics [62]. Conversely, oral intake of contaminated soil is the main route for crows. Hack and Selenka [63] suggested that the action of enzymes on alimentary lipids and the potential of bile to form mixed micelles with fatty acids and monoglycerides enhance xenobiotic mobilization to a high degree in a gastrointestinal model. Such gastrointestinal absorption could contribute to higher $BCF_{measured}$ than $BCF_{theoretical}$. Higher organic content in particles can result in strong binding of 2,3,7,8-T_4CDD onto the particle, lowering its bioavailability [64]. When K_{pw} was calculated, sediment was substituted for the particle phase [61]. The sediment might contain higher organic content than soil from the DS. Higher K_{pw} leads to lower $BCF_{theoretical}$. Furthermore, Stephens et al. [65] reported that PCDD/Fs in soils ingested by chicken are readily absorbed and are bioaccumulated in the tissues. Their estimated BCFs ($BCF_{chicken}$) of PCDD/Fs from

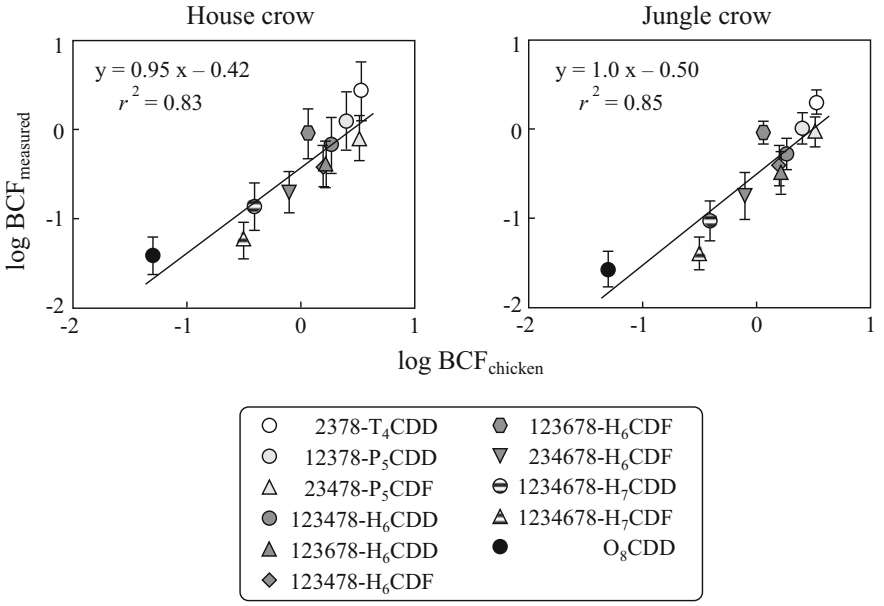

Fig. 13 Relationships between log $BCF_{measured}$ and log $BCF_{chicken}$ for PCDD/Fs. Each plot and bar means average and standard deviation, respectively. Data of $BCF_{measured}$ and $BCF_{chicken}$ were cited from Watanabe et al. [58] and Stephens et al. [65], respectively

soils to thigh muscle after feeding a diet mixed with 10% of highly contaminated soil for 80 days were generally consistent with our data (Fig. 13), supporting that crows in the DS consume soil with raw garbage.

Lower BCFs for congeners with larger molecules such as H_7- and O_8-CDDs/Fs were shown in Figs. 12 and 13. This may be due to the lower uptake efficiency of these congeners through the gastrointestinal tract. A previous study for wild tufted ducks reported that biomagnification factors (BMFs) of PCDD/F congeners tended to decrease with their K_{ow} [66]. In humans, the net absorption of PCDD/F congeners is likely to be diminished with the degree of chlorination [67]. Mean log BCF values in the DS crows had significant negative correlations with log K_{ow} and molecular weight of PCDD/F congeners (Fig. 14). Opperhuizen and Sijm [68] pointed out a lack of membrane permeation for hydrophobic chemicals with widths over 0.95 nm. In wild common cormorants, congeners with large molecules such as H_7CDD/F and O_8CDD showed no life-stage-dependent accumulation probably because of gastrointestinal barrier, whereas T_4- to H_6-CDDs and P_5- and H_6-CDFs showed significant increase with growth [27]. The results shown in Fig. 14 clearly demonstrate that molecular configuration may limit the dietary uptake of PCDD/F congeners in crows.

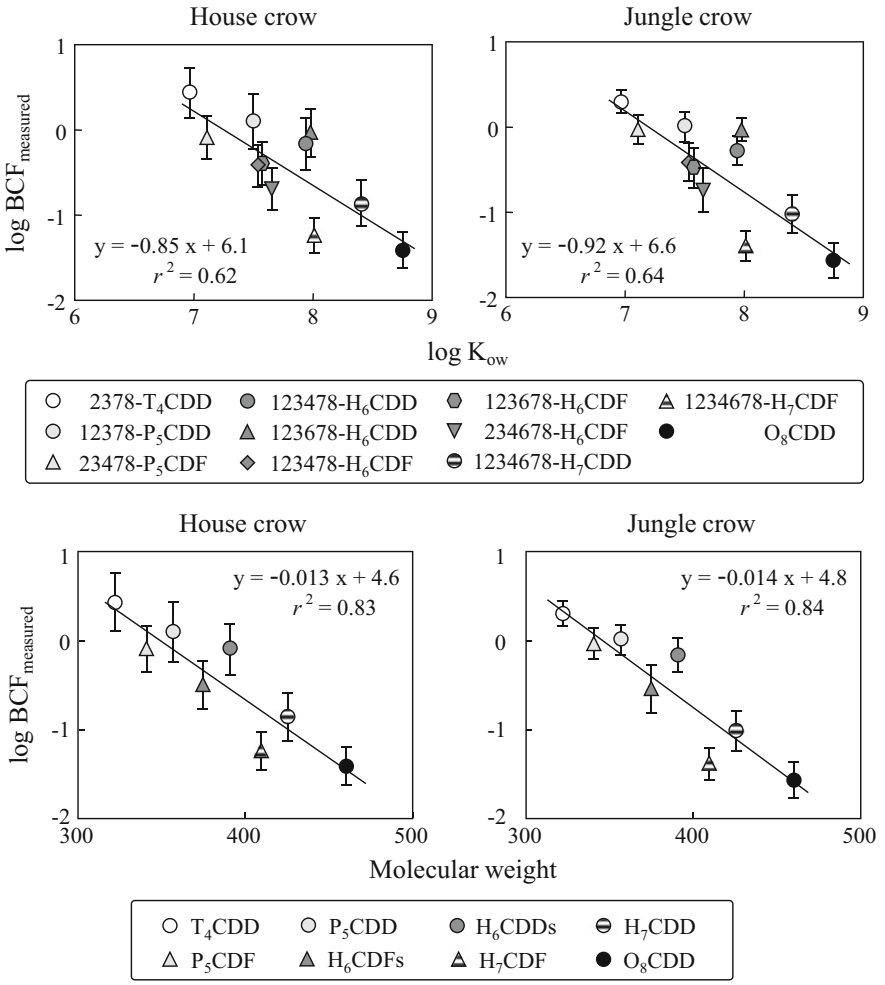

Fig. 14 Relationships between log $BCF_{measured}$ for PCDD/Fs in crows from the DS and log K_{ow} or molecular weight of each congener. Each plot and bar means average and standard deviation, respectively. Data were cited from Watanabe et al. [58]

5.2 Pig

5.2.1 Accumulation Features

As in the case of crows described above, pigs inhabiting the DS in Chennai also feed on raw garbage with contaminated soils (Fig. 11), and hence considerable exposure to DRCs and adverse effects on their health are expected. Our group recently examined exposure levels, accumulation features, and toxicological effects of DRCs by analyzing samples of liver, abdominal fat, muscle, and plasma

collected from pigs in the DS and a RS in Chennai [59]. Concentrations of DRCs in tissue samples from the DS pigs were significantly higher than those from the RS. In addition, similar DRC congener profiles between pig tissue and soil from the DS were shown. These observations support that wild animals, such as crows and pigs, in the DS are highly exposed to these contaminants through ingestion of on-site garbage contaminated with soil.

5.2.2 Relationships with Hepatic Cytochrome P450 Enzymes

Availability of fresh liver from the pigs enabled the measurement of cytochrome P450 (CYP) 1A1, CYP2B1, and CYP4A1 by immunoblotting assays. A single band of protein cross-reacted with each antibody around the corresponding rat CYP standard was detected in pig hepatic microsomes (Fig. 15). When the relationships between TEQs and CYP1A-, CYP2B-, or CYP4A-like protein levels were examined in the pig liver, hepatic TEQs (wet weight basis) were positively correlated with the levels of CYP1A-like protein ($p < 0.05$, Fig. 16). Induction of CYP1A enzymes through the aryl hydrocarbon receptor (AhR) has been used extensively as a sensitive indicator of exposure and effects of DRCs. Schmitz et al. [69] and Zeiger et al. [70] reported no-observed-effect level (NOEL) and half-maximum induction (EC_{50}) values for TCDD-induced EROD in HepG2 cells, H4IIE cells, and Wistar rat primary hepatocytes; the EC_{50} values were 220, 16, and 6.4 pg/ml, and NOEL values were 11, 0.064, and 0.013 pg/ml, respectively. TEQs (mean $\pm$ SD: 3.9 ± 3.2 pg/g wet weight) in the liver of all pigs from India were higher than the NOELs for EROD in H4IIE and rat primary hepatocytes, but were lower than that in HepG2. Silkworth et al. [71] reported that human hepatocytes are about 10–1,000 times less sensitive for CYP1A induction by certain DRC congeners than rat and monkey cells. These observations suggest that pigs may be more sensitive to

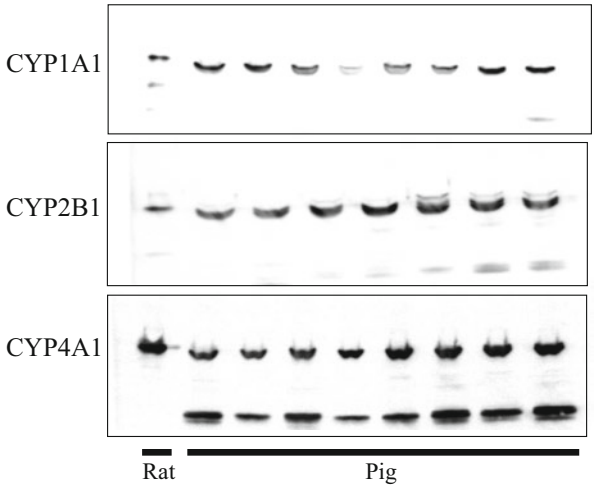

Fig. 15 Results of immunoblot analyses of pig hepatic microsomes using anti-rat CYP1A1, CYP2B1, and CYP4A1 polyclonal antibodies. Data were cited from Watanabe et al. [59]

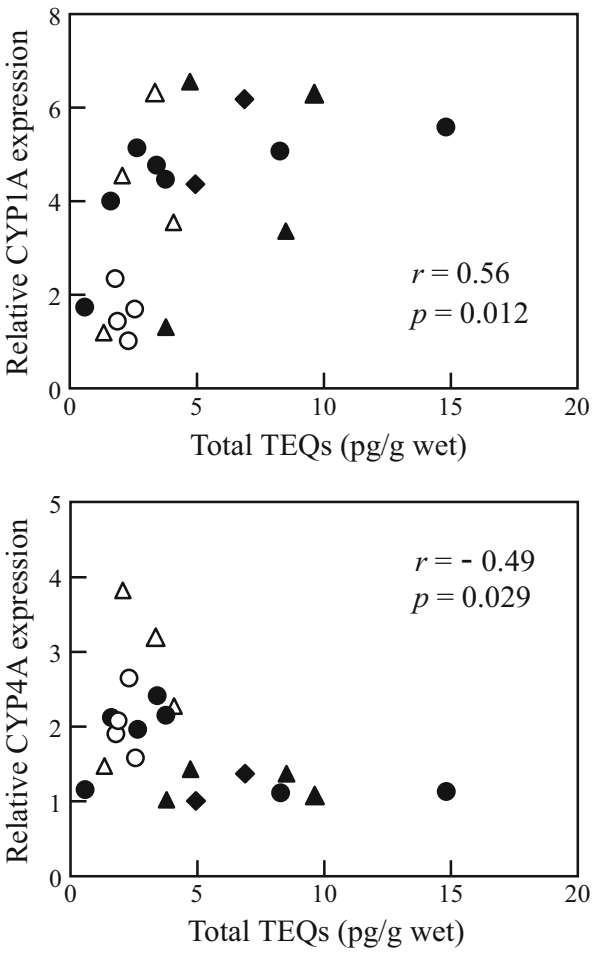

Fig. 16 Relationships between hepatic TEQs and expression levels of CYP1A- or CYP4A-like protein in the liver microsomes of pigs from India. *Filled circle* = female from the dumping site; *filled triangle* = male from the dumping site; *open circle* = female from the reference site; *open triangle* = male from the reference site; *filled diamond* = piglets from the dumping site. Data were cited from Watanabe et al. [59]

CYP1A induction by DRCs than humans. On the other hand, CYP4A-like protein content was negatively correlated with TEQ levels in the pig liver ($p < 0.05$, Fig. 16), whereas CYP2B-like protein revealed no correlation with hepatic TEQs. The negative correlation between hepatic CYP4A-like protein and TEQ levels is consistent with a previous study which reported the suppression of the CYP4A protein in rat treated with AhR ligand [72]. Koga et al. [73] demonstrated decreased hepatic CYP4A1 expression in rat treated with CB-77. Given that CYP4A1 is induced through peroxisome proliferator-activated receptor α (PPARα), it is likely that DRCs have a potential to affect PPARα-signaling pathways in pigs. Disruption of PPARα-signaling pathway may pose health hazards, as PPARα is involved in development, physiology, and inflammatory response [74].

5.2.3 Hepatic Sequestration

On the lipid-weight basis, the concentrations of PCDD/F and non-*ortho* DL-PCB congeners in the liver were higher than those in the adipose and muscle tissues of pigs, while the mono-*ortho* DL-PCB congener levels were almost similar among these different tissues. This result suggests the lipid-dependent accumulation of mono-*ortho* DL-PCB congeners and the specific binding of PCDD/F and non-*ortho* DL-PCB congeners to proteins. Table 2 shows liver/adipose concentration ratios (L/A ratios on lipid-weight basis) and their relationship with hepatic CYP1A-like protein content. L/A ratios of most PCDD/F congeners were significantly positively correlated with CYP1A-like protein content ($p < 0.05$), indicating that CYP1A is involved in the hepatic sequestration of these congeners. The ratios for all mono-*ortho* DL-PCB congeners were near 1.0, which means no hepatic sequestration of these congeners. As for PCDD congeners, the L/A ratios increased with an increasing number of chlorine substitutions. Similar trends for PCDD/F congeners were reported in our earlier investigations on wild animals [27, 28, 75–77]. A possible mechanism to explain dose-dependent hepatic sequestration is the induction of hepatic microsomal protein, CYP1A2, and the subsequent binding of PCDD/F congeners to this protein. Comparisons between DRC-dosed CYP1A2 knockout and parental strains of mice provided direct evidence that CYP1A2 was the target protein for the binding of 2,3,7,8-T_4CDD, 1,2,3,7,8-P_5CDD, and 2,3,4,7,8-P_5CDF in the liver, but not for CB-153 [78]. Interspecies comparison of the L/A (or liver/muscle) ratios showed that the capacity of hepatic sequestration of DRCs in pigs was comparable to that in raccoon dog [28], but higher than those in Baikal seal [75], common cormorant [27], and jungle crow [77].

5.2.4 Maternal Transfer

To understand the maternal transfer of DRC congeners in pigs, the hepatic concentrations of DRCs in a dam-piglets pair (two piglets and their dam) from the DS were measured and compared. Concentration ratios of DRCs between piglets and their dam (piglets/dam) exceeded 1.0, and especially the congeners with a molecular weight between 360 and 400 were detected at higher concentrations in piglets than in their dam (Fig. 17); this shows maternal transfer of DRCs. Such transfers of DRCs from dams to neonates via milk have been considered to be more significant than placental transport [79]. Iwata et al. [75] showed significant declines in 2,3,7,8-T_4CDD, 1,2,3,7,8-P_5CDD, 2,3,4,7,8-P_5CDF, CB-126, CB-169, and CB-157 concentrations with age in the liver of wild female Baikal seals, suggesting that these congeners are easily eliminated through lactation. Low molecular weight congeners with a molecular weight less than 360, however, might be metabolized by the piglets' hepatic CYP1A, whose expressions were as high as in the adults (Fig. 16). This would explain lower concentration ratios (piglets/dam) for lower molecular weight congeners than for those with 360 to 400 molecular weights.

Table 2 Liver to adipose concentration ratios (L/A, lipid basis) of DRCs and their relationships with hepatic CYP1A-like protein contents in pigs from India

Congener	n[a]	L/A concentration ratio		Relationship between CYP1A and L/A	
		Mean	Range	S[b]	r^2
PCDDs					
2,3,7,8-T_4CDD	3	5.1	2.2–7.8	na	na
1,2,3,7,8-P_5CDD	7	14	0.43–63	10	0.61*
1,2,3,4,7,8-H_6CDD	9	21	2.9–55	8.2	0.62*
1,2,3,6,7,8-H_6CDD	12	19	0.47–83	8.5	0.39*
1,2,3,7,8,9-H_6CDD	4	18	4.4–31	na	na
1,2,3,4,6,7,8-H_7CDD	12	55	2.8–210	21	0.35*
O_8CDD	12	91	4.2–210	23	0.44*
PCDFs					
2,3,7,8-T_4CDF	4	4.2	2.2–7.0	na	na
1,2,3,7,8-P_5CDF	0	na	na	na	na
2,3,4,7,8-P_5CDF	11	130	3.9–400	62	0.66**
1,2,3,4,7,8-H_6CDF	11	73	1.7–270	37	0.61**
1,2,3,6,7,8-H_6CDF	9	93	2.4–320	42	0.57*
1,2,3,7,8,9-H_6CDF	0	na	na	na	na
2,3,4,6,7,8-H_6CDF	5	85	5.2–190	39	0.45
1,2,3,4,6,7,8-H_7CDF	12	120	4.3–360	44	0.54**
1,2,3,4,7,8,9-H_7CDF	1	55	55–55	na	na
O_8CDF	7	360	13–1,000	140	0.35
Non-ortho DL-PCBs					
PCB-77	12	7.5	0.35–77	1.2	0.008
PCB-81	10	8.7	2.3–25	2.3	0.16
PCB-126	12	24	2.4–110	9.9	0.32
PCB-169	12	2.7	0.73–10	0.77	0.24
Mono-ortho DL-PCBs					
PCB-105	12	1.7	0.24–10	−0.17	0.010
PCB-114	12	0.82	0.26–2.9	0.0017	<0.001
PCB-118	12	1.0	0.24–4.2	0.0061	<0.001
PCB-123	9	1.0	0.35–3.0	−0.12	0.026
PCB-156	12	0.73	0.36–1.6	−0.0031	0.013
PCB-157	12	0.85	0.39–1.9	0.028	0.009
PCB-167	12	0.89	0.21–1.9	0.19	0.38*
PCB-189	12	0.81	0.14–1.9	0.077	0.047

[a]The number of specimens in which the congener was detected both in the liver and adipose tissue
[b]S = the slope of the following equation; Concentration ratio (liver/adipose) = (CYP1A-like protein content) × S + intercept. This calculation was done when n was 5 or more
*$p < 0.05$, **$p < 0.01$; *na* no data available

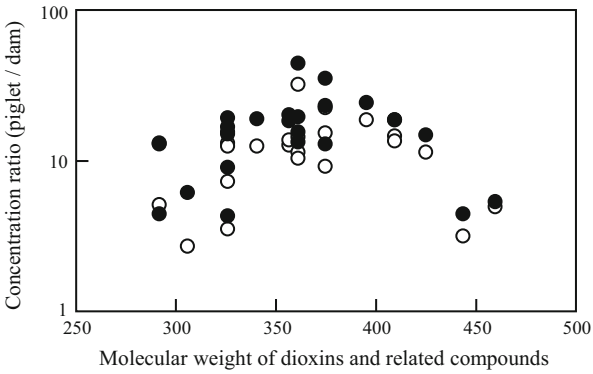

Fig. 17 Relationship between molecular weight of dioxins and related compounds (DRCs) and concentration ratios (piglets/dam) of DRCs in the liver of pigs from the dumping site. *Solid and open circles* represent different piglets whose mother is the same individual. Data were cited from Watanabe et al. [59]

Iwata et al. [75] also reported poor elimination of H_7CDD to O_8CDD congeners in aged mothers, suggesting less excretion of such highly chlorinated congeners through lactation. Data from Van den Berg et al. [80] also support the results, showing decreased excretion rates via dam milk with increasing chlorine content.

5.2.5 Biochemical Effects

To assess the biochemical effects in pigs inhabiting the DS, concentrations of plasma hormones, immunoglobulins, and vitamin A were measured and compared to those in the RS pigs. Interestingly, plasma immunoglobulin G (IgG) levels were significantly lower in male pigs from the DS than those from the RS ($p < 0.05$, Fig. 18). The immune system is one of the most sensitive targets of 2,3,7,8-T_4CDD [81], and plasma IgG is reported to be suppressed by 2,3,7,8-T_4CDD exposure through the suppression of antigen-responding B-cell proliferation during germinal center formation in mice [82]. In humans from Seveso, Italy, plasma 2,3,7,8-T_4CDD concentrations (3.5–90 pg/g lipid) were negatively associated with plasma IgG concentrations [83]. TEQs in pigs (170 ± 87 pg/g lipid in males and 110 ± 110 pg/g lipid in females) from the DS were similar to those reported for the Seveso population, indicating that DRCs may affect the immune system in the DS pigs. A significant difference between the DS and RS was also observed for plasma-free thyroxine (FT_4) levels in females (Fig. 18). When all specimens were analyzed together (plasma levels of FT_4 had no significant difference between gender), a decrease in plasma FT_4 in the pigs from the DS was detected ($p = 0.039$), compared with those from the RS. The competitive binding of DL-PCBs and T_4 to transthyretin and glucuronidation of T_4 by dioxin-inducible UDP-glucuronyl transferase may account for the decrease of FT_4 in the DS pigs. Correlation analyses between hepatic TEQs and plasma hormone levels showed no specific patterns.

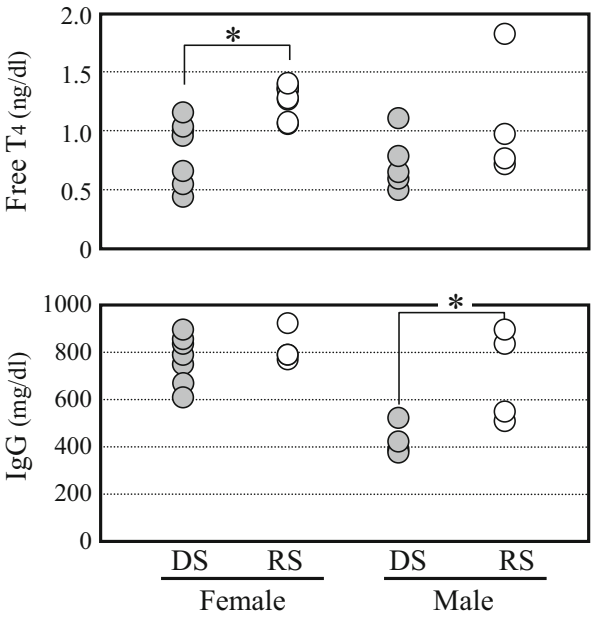

Fig. 18 Comparison of plasma-free thyroxine (T_4) and immunoglobulin G (IgG) levels between sampling sites in the pigs from India. DS and RS represent dumping and reference sites, respectively. $^*p < 0.05$

5.2.6 Hydroxylated Metabolites

We have recently analyzed hydroxylated metabolites of PCBs (OH-PCBs) in the blood of pigs from India and found higher concentrations of OH-PCBs in the DS pigs, especially piglets, than in the RS pigs [84]. In addition, OH-PCB concentrations in the blood were positively correlated with hepatic CYP1A-like protein content ($p < 0.01$), indicating the CYP1A-dependent formation of OH-PCBs in the liver and the subsequent retention of these metabolites in the blood of pigs. Considering that hepatic levels of DRCs and CYP1A expression were higher in the DS pigs, as described earlier, OH-PCBs in the DS pigs could be preferentially formed from PCBs through CYP1A-mediated metabolism induced by DRC exposure. Thus, DRCs in the liver of DS pigs pose effects on hepatic CYP1A and CYP4A expression and are probably sequestered by the induced CYP1A protein, and subsequently hydroxylated metabolites of xenobiotic chemicals including OH-PCBs are formed. Plasma IgG and T_4 levels may also be affected by DRCs accumulated in the DS pigs. Swine is considered as a prospective model animal to predict bioavailability and biotransformation of environmental chemicals in humans, due to the similarities of gastrointestinal tract function [85], nutritional requirements [86], and CYP activities [87]. The similar phenomena observed in the pigs from the Indian DS may arise in the residents living around the DS, and hence more attention should be paid for the human risk from not only DRCs but also hydroxylated metabolites, which are formed by DRC-induced CYP1A protein. Management of the DS is crucial to protect the health of the inhabiting wild animals and humans.

6 Conclusions and Future Consideration (E-Wastes)

Recent studies have demonstrated that DS in Asian developing countries is a potential source of DRCs. Levels, profiles, and estimated fluxes of DRCs observed for soils in DS suggested that these contaminants are formed by uncontrolled burning of solid waste, and DS in India and the Philippines may be a significant reservoir for DRCs. In India, the concentrations of DRCs in human breast milk from two DS in Chennai and Kolkata were significantly higher than those from RS and other Asian developing countries, and elevated levels of these contaminants were observed in bovine milk collected from buffalo and cows feeding in the Chennai DS and in fish collected from a pond adjacent to the Kolkata DS. These results indicate that residents around these DS have been exposed to considerably high levels of DRCs, probably through the intake of contaminated bovine milk and fish. Wild crows and pigs inhabiting the DS in Chennai were also contaminated by DRCs, and direct transfer of these contaminants from contaminated soil was suggested. In addition, the study on pigs suggested that DRCs pose effects on hepatic CYP1A and CYP4A expression and are probably sequestered by the induced CYP1A protein. Plasma IgG and T_4 levels may also be affected by DRCs in pigs from the DS. Because there is no control and measure of DRC release in DS, it can be anticipated that pollution by DRCs will become exacerbated further. In view of these observations, we suggest that further investigations on the pollution sources and animal exposure of DRCs in Asian developing countries, especially DS, are needed to elucidate future pollution trends and to assess the health risk to humans and wildlife.

In recent years, increasing activities of electrical and electronic waste (e-waste) recycling in developing countries have received international attention, because of the emission of toxic chemicals resulting from the uncontrolled recycling processes of e-waste facilities. In Asia, environmental fate and human exposure of hazardous substances released from e-waste recycling sites (EWRS) have been extensively investigated in China, where e-waste recycling plays an important economic role, since 2000. EWRS have been identified as hotspots of not only polybrominated diphenyl ethers (PBDEs) which are contained in e-wastes as flame retardants but also DRCs such as PCDD/Fs and polybrominated dibenzo-*p*-dioxins/furans (PBDD/Fs) [88]. Despite the presence of EWRS in developing countries, researches on DRCs in EWRS are exceedingly limited in Asian countries other than China. Our group has recently found significantly higher concentrations of PCDD/Fs, PBDD/Fs, and DL-PCBs in dust from two EWRS in North Vietnam compared with those from an urban site (Hanoi) and suggested a substantial release of these DRCs by recycling activities [89]. Furthermore, dioxin-like activities in the extract of EWRS dust, estimated using the dioxin-responsive chemically activated luciferase gene expression (DR-CALUX) assay, were also greater than those in the urban dust, and higher percentage of unknown dioxin-like activities was observed in the dust extract, indicating large contribution from unidentified DRCs (other than PCDD/Fs, PBDD/Fs, and DL-PCBs) [88]. Given the above results, the role of "e-wastes" as a significant source of DRCs to the environment should be urgently elucidated in Asian developing countries.

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Risk Assessment for Dioxins and Related Compounds

Martin Rose

Abstract The presence of dioxins and polychlorinated biphenyls (PCBs) and other ubiquitous environmental contaminants in food and feed is generally unavoidable. As a consequence, human and animal exposure to these compounds is also unavoidable. We know that some of these compounds are highly toxic. It is therefore important to be able to establish whether or not these substances, at the levels they are found, are likely to cause adverse health effects in either animals or humans. Risk assessment consists of hazard identification, hazard characterisation, exposure assessment and risk characterisation. The aim of a risk assessment is to set a health-based guidance value to determine an intake that may be judged to be without appreciable risk or to identify a margin of exposure between a reference point or point of departure (in the case of North America) associated with a corresponding dose–response curve and estimated exposure. Risk management relies heavily on the outcome of the risk assessment process but will also take into account socio-economic and political factors.

Keywords Dioxins, Exposure assessment, Hazard characterisation, Intake, Risk assessment

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Contents

1 Background

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDDs and PCDFs; 'dioxins') are ubiquitous in the environment and are always present in food and human tissues, even when there is no history of occupational or accidental exposure. Exposure can sometimes occur through inhalation of air, dermal absorption, consumption of drinking water and consumption of food, but there is no doubt that it is food that is the predominant route for the vast majority of the population [1].

Of the 75 PCDD and 135 PCDF congeners, only the 17 that contain chlorine at the 2,3,7 and 8 positions persist and accumulate in animal and human tissues [2]. These congeners form only a small proportion of the environmental load, since many sources of pollution will release the full range of congeners. These 2,3,7,8-substituted congeners are proven to be the most toxic and are thus the primary focus of interest. They are highly lipophilic and are found primarily in fatty tissues such as lipid-rich food.

Besides 2,3,7,8-substituted PCDDs and PCDFs, it has been established that some PCBs also bind to the Ah-receptor and elicit dioxin-like biochemical and toxic responses. Thus, the assessment of the health risks of exposure to dioxin-like chemicals must consider these PCBs as well as PCDD/Fs. PCBs have a variety of other non-dioxin-like effects. However, the risk assessment of 'dioxins' is incomplete without the inclusion of dioxin-like PCBs. Nevertheless, other non-AhR-related effects of PCBs or their metabolites should also be considered to get a full picture of the actual risks for humans and wildlife.

In 1987, Travis concluded from modelling calculations that the dietary intake of 2,3,7,8-TCDD accounted for 98% of human exposure to this compound [3]. Over the last 15 years, many measurements have been made of the full range of PCDD/Fs in foods, and many estimates of exposure have been made which all lead to the conclusion that well over 90% of total exposure for most people originates from the diet [4, 5]. For this reason, the rest of this chapter is focussed on the risk assessment of dietary exposure to PCDD/Fs and PCBs.

Dioxins and PCBs in food can often originate from animal feed. This feed is a low-cost commodity and contamination incidents have been caused by the use of contaminated ingredients or poor manufacturing processes or even malicious addition of nonfood (industrial) oils.

In addition, a number of other classes of contaminants also add to the total number of compounds that have a dioxin-like mode of action. These include brominated and mixed halogenated dioxins, furans and biphenyls, polychlorinated (and brominated) naphthalenes and larger polycyclic aromatic hydrocarbons (PAHs) substituted with halogens in which specific chlorination patterns play an important role.

2 Toxic Equivalency Factors

Because of the need to assess the risk from complex mixtures of PCDD/Fs, the approach that is now globally accepted assigns relative potency factors to each congener, based on a comparison with the potency of 2,3,7,8-TCDD, which serves as a reference compound [6]. In this concept, each congener is assigned a toxic equivalency factor (TEF) relative to 2,3,7,8-TCDD. The total toxic equivalency of a mixture of PCDDs, PCDFs and PCBs is the sum of the TEFs multiplied by the concentrations of each compound in the (food) matrix, to give a toxic equivalence (TEQ). However, there is some variation in the terminology used by different authors; total TEQ, summed TEQ and ΣTEQ are self-explanatory, whilst the less explicit acronym, TEQ, is used by different authors to refer either to the total or to the TEF × concentration product of an individual congener.

Although consideration of total TEQs is essential, it is unfortunate that many data are made available only in this form without the make-up of contributing congeners. Total TEQ figures do not give a full picture, and their interpretation is made possible by inspection of the underlying congener-specific data, especially when data from different laboratories are compared. The contribution of specific congeners to the total is also of great value for source identification. Historically, the main reason that congener-specific details have not been given has been due to journal limitations regarding the length of papers submitted. But in more recent times, many journals are allowing the submission of supplementary information in electronic format. Therefore, authors should be encouraged to submit congener-specific data in this way in order to allow a more thorough inspection of data for later evaluation or to archive results in publically available electronic databases or

with public bodies, e.g. the European Food Safety Authority (EFSA) – an issue that will be addressed later in this chapter.

There remains some uncertainty about the accuracy with which TEF values reflect the actual potential for health effects on humans. It should be noted that TEF values are given only to the nearest one-half order of magnitude +/− half a log and therefore contribute significantly to uncertainties in risk assessment.

It was recognised in the 2005 TEF revision [6] that other classes of compounds have the same mode of toxic action, and it was recommended that they should be included in future TEF revisions. For example, a preliminary TEF assessment has been made of brominated dibenzodioxins, dibenzofurans and biphenyls. As a result, it has been recommended by the WHO/UNEP that these compounds should be assigned the same TEFs as their chlorinated analogues as an interim measure for human risk assessment [7].

2.1 Previous TEF Schemes for PCDDs and PCDFs

During the 1980s, a rather large number of different TEF schemes were used. International toxic equivalency factors (I-TEFs) for PCDD/Fs were set in 1990 [8] and were adopted by almost all scientists and regulatory authorities at that time. A more recent system of TEFs, set by the World Health Organization in 1997 (WHO-TEFs) [9–11] and revised to take into account new data in 2005 [6, 7], has since been accepted by most authorities.

2.2 Inclusion of PCBs into TEF Scheme

The WHO system also sets TEFs for those PCBs that bind to the Ah-receptor and elicit dioxin-like biochemical and toxic responses. These congeners have at least two adjacent meta-/para-chlorines and not more than one chlorine substituted in the ortho-position. In 1994, TEFs were set for three non-ortho-PCBs (IUPAC nos. 77, 126 and 169), eight mono-ortho-PCBs (105, 114, 118, 123, 156, 157, 167 and 189) and for the di-ortho-PCBs 170 and 180 [11]. In the 1997 WHO scheme [9], PCBs 170 and 180 were removed, PCB 81 was added and the TEF for the non-ortho-PCB 77 was reduced by a factor of 5. For most food samples, these changes make only a small difference to the total TEQ attributable to PCBs, although for some congeners, e.g. PCB 153 which is present in relatively high quantities, the impact can be more significant.

The term coplanar PCB is often used to refer to the three non-ortho-compounds and sometimes to these and the eight mono-ortho-PCBs listed above. In many food samples, the TEQ contribution made by these dioxin-like PCBs may equal or, especially in fish, exceed that made by the 2,3,7,8-substituted PCDDs and PCDFs.

3 The Risk Assessment Process and Definitions

Risk assessment is defined by IPCS as 'a process intended to calculate or estimate the risk to a given target organism, system or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system' [12]. When considering chemicals in food, the term safety assessment is sometimes used, where the term safety is the 'practical certainty that adverse effects will not result from exposure to an agent under defined circumstances'. Risk is defined as the 'probability of an adverse effect in an organism, system or (sub)-population caused under specified circumstances by exposure to an agent' [12, 13].

The risk assessment process is science or evidence based and is a well-established approach designed to protect humans and the environment. It forms part of the overall risk analysis that also covers risk management and risk communication. It is increasingly seen as important to separate the activities of risk assessment process from that of risk management. This is to ensure the scientific independence and objectivity of risk assessment which should be solely evidence based. This is an important difference with risk management and communication that also need to take into account political and socio-economic considerations [13].

Risk managers will nevertheless need to communicate and interact with risk assessors during the risk assessment process, particularly during the initial problem definition phase ('framing the question') to ensure that the output of the risk assessment is indeed useful for them. Best outcomes are therefore achieved when the relationship between risk assessment and risk management is a dynamic, interactive, often iterative, process (Fig. 1) [13].

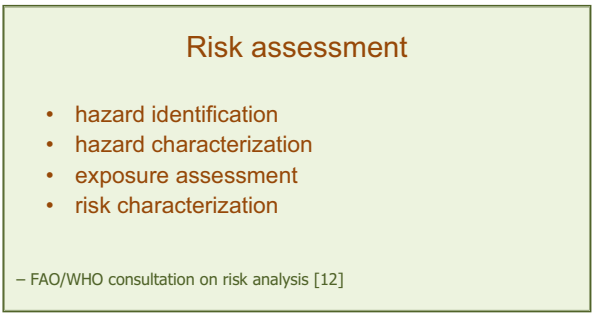

Fig. 1 The key stages in the risk assessment process

3.1 Hazard and Exposure

Risk is determined by both the hazard characterisation and the exposure assessment. If exposure is below a level that is toxicologically relevant and taking into account specific species and individual sensitivities, there will be no expected risk. The higher the exposure, the greater the risk. In chemical risk assessment, hazard is defined as an 'inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)population is exposed to that agent'. This differs from microbiological risk assessment, wherein the hazard is generally considered to be the biological agent, rather than its properties. There can be a number of different hazards associated with an individual chemical, influenced by its mode(s) of action along with the route, the magnitude and duration of exposure and the exposed population (e.g. different life stages). The most relevant endpoint can be, for example, cancer for lifelong exposure or effects on reproduction and development. Usually the most sensitive relevant endpoint is taken for risk assessment.

3.2 Tolerable Intakes

The presence of dioxin-like compounds in food is unavoidable, and consequently zero human exposure is not possible. The primary aim of the risk assessment is to establish an exposure level at which there is no appreciable risk to, for example, human health. The overall term for this is the health-based guidance value (HBGV) and this covers more specific values such as the tolerable daily intake (TDI), tolerable weekly intake (TWI), provisional tolerable monthly intake (PTMI) and similar terms [14]. To establish an HBGV, account needs to be taken of all available toxicological information, including studies on humans, experimental animals, cell-based systems and any other data. Only a few cases of human occupational or other exposure exist and so the HBGV is usually based on data from repeated dose studies conducted on experimental animals, such as chronic (or sub-chronic) toxicity or multi-generation studies in rats and mice. To establish an HBGV, a reference point (RP) or point of departure needs to be identified, based on the mathematical modelling of the dose–response relationship. The reference point can vary but for example it is possible to use a benchmark dose lower confidence limit (BMDL) as the RP. This is an estimate of the lowest dose that is 95% certain to cause no more than a specified change when compared to a background. If there is insufficient data or other reason why modelling is not appropriate, another RP may be used, such as the no-observed-adverse-effect level (NOAEL), which is the highest dose not to cause a significant adverse effect when compared with a control population. The HBGV is established by dividing the RP by uncertainty (or safety) factors to account for the extrapolation from animals to humans and for human-to-human variability in terms of sensitivity [14].

3.3 Uncertainty (Safety) Factors

A default uncertainty factor of 100 has been used for many risk assessments. The value was derived in an arbitrary way, but it was soon recognised that it comprised of at least two components:

1. A factor of 10 to account for interspecies differences, i.e. to allow for possible greater sensitivity of humans compared with the animal model, due to slower elimination from the body, greater balance of activation to detoxification reactions and/or greater sensitivity to the toxic effect
2. A factor of 10 to account for human interindividual (intraspecies) variation, i.e. the possibility that a proportion of the population may be at greater risk because of differences in toxicokinetics or tissue sensitivity within the human population [13].

The overall uncertainty factor of 100 may be increased if there are important gaps in the underlying toxicological database for a compound, e.g. the absence of a NOAEL or of long-term animal studies. If the derivation of a TDI is based on human data, then it may be deemed that the uncertainty factor may be absent or less than a factor of 10 [13].

More recently there have been approaches to refine the uncertainty factor by further subdividing the tenfold factors into factors for the toxicokinetic and toxicodynamic aspects. Examination of various databases has indicated a differential split, with greater weight given to toxicokinetic causes of interspecies differences, whereas equal weighting may be given to toxicodynamic and toxicokinetic differences in individual variability (Fig. 2). If individual data on any of these components are available, they could then be incorporated into the evaluation by the replacement of the corresponding default factors of 10. For example, if information is available indicating that the toxicokinetics of a particular chemical are

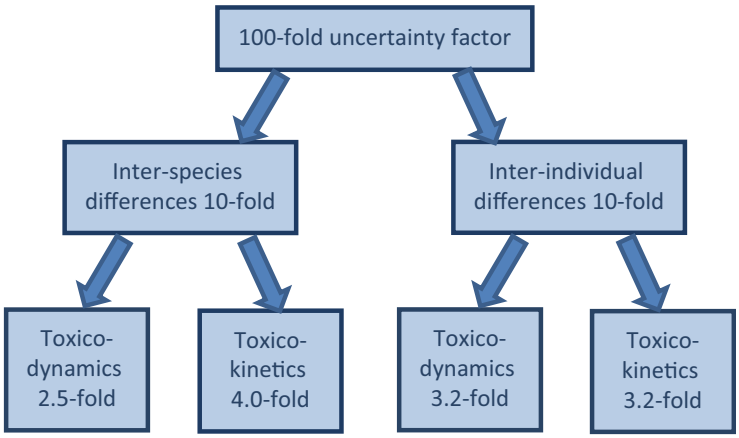

Fig. 2 Subdivision of uncertainty factors [13, 15]

quantitatively similar in the experimental animal used to establish the NOAEL and in humans, then the default factor of 4.0 in Fig. 2 would be replaced by the value of 1. The factors would then be 2.5 for interspecies differences in toxicokinetics and 10 for human variability, giving an overall factor of 25.

For most chemicals, the default safety factors seem appropriate for adequate protection. However, where data on a compound indicate that the defaults are inappropriate (too low or too high), then the subdivision of the factors allows additional data to be used to modify the defaults and introduce compound-specific data. The latter is the case for dioxin-like compounds and modified uncertainty factors and chemical-specific adjustment factors were used for the derivation of a TDI by, for example, the WHO and the EU [16]. A similar approach has been used, for example, for methylmercury [17] and zearalenone [18].

During the 1980s and early 1990s, a number of countries performed risk assessments and derived tolerable daily intakes (TDIs) of dioxins in the range of 1–10 pg/kg bw/day, as reviewed by Larsen et al. [19]. A TDI is defined as the maximum amount of a contaminant, to which an individual can be exposed every day over a whole lifetime without incurring appreciable risk to health. Note here the use of terminology such as tolerable and appreciable. These (often unquantified) aspects already have a political and socio-economic aspect, emphasising the close working to agreed protocols that is necessary between the risk assessor and the risk manager.

As the data on aspects of the toxicology of PCDD/Fs and PCBs has become more extensive and of better quality during the last two decades, views about the appropriate value of a TDI have changed, and values resulting from different assessments have become more consistent.

Thus, whilst in 1990 the WHO established a TDI of 10 pg/kg bw/day for TCDD, in 1998, an expert consultation concluded that the TDI was in the range of 1–4 pg TEQ/kg bw/day [20]. In May 2001, the Scientific Committee on Food (SCF), an expert committee that advised the European Commission prior to the establishment of EFSA, concluded that the tolerable intake should be expressed on a weekly rather than a daily basis and set a TWI of 14 pg WHO-TEQ/kg bw/week [16]. The WHO/FAO Joint Expert Committee on Food Additives (JECFA) established, in June 2001, a PTMI of 70 pg/kg bw/month [21]. There are also a number of HBRVs such as the one established by the UK independent Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) of 2 pg TEQ/kg bw/day [22].

The tendency to move from day to week to month reflects the fact that the in vivo half-life of dioxins is very long (measured in decades) and so body burdens build up very slowly. Exceeding a TDI or TWI or even a TMI value by a small amount for a short period of time is not necessarily a cause for concern.

3.4 Food Monitoring and Surveys

Exposure to chemicals from the diet is normally assessed by food surveys or monitoring programmes. Surveys can be relatively simple and directed towards particular food types for specific compounds of concern, for example, dioxins in fish. They can also be more complex and directed towards gathering data for population exposure estimates and can take the form of, for example, total diet studies (TDS), duplicate diet studies or market basket surveys.

The characteristics of TDS surveys are that the food is analysed as it would be eaten (e.g. after cooking) and that individual food items are pooled and analysed as a composite. The sole purpose of pooling is to get information on many samples but at a reduced cost. The degree of pooling is driven by the purpose of the survey and funding available. TDS surveys are particularly well suited for dioxins where the cost of the analysis is quite high in comparison to most other chemical contaminants. A TDS can be used for screening purposes, analysing a limited number of broadly pooled food samples. Alternatively, a more refined TDS can include the analysis of a greater number of less pooled samples, often separately covering different seasons and regions. The total diet study as described by Harris [23], Buss and Lindsay [24] and Peattie et al. [25] categorises food into groups such as milk, eggs, fish, etc., based on their relative importance in the diet. Individual samples of retail food from these groups are purchased at regular intervals from locations within an area (typically a country) selected at random but weighted according to population density. They are prepared for consumption and combined to form single composite samples for each of the defined food groups. Such an analysis of a year's combined samples ensures that a large, diverse base of individual samples sourced from across the region of study is included and that they represent average dietary habits. It will also allow temporal trends to be studied. TDS exercises have been carried out for dioxins and PCBs [26]. Although the total diet study approach as described here for the United Kingdom is used in some other countries/regions, e.g. the Basque region in Northern Spain [27, 28], it is more commonplace to use a market basket approach. This is described below and consists of samples collected from various food categories, followed by cooking and preparation as for human consumption. This is more properly called a refined TDS.

A duplicate diet study involves volunteers who collect a replicate sample of all food consumed over a given period. The samples from each individual are made into a single pooled sample, and these can be used to look at intake variation between individuals. Care has to be taken with such studies, because the behaviour of some volunteers can change for the duration of the study and the diets collected do not always represent a typical diet. This approach was used for example in a study by Arisawa et al. on dioxins and PCBs in the Japanese population [29]. It has also been used for a much wider range of food chemicals for example in a study by Clarke et al. where phytooestrogens, trace elements, natural toxicants and nitrates were measured in summer and winter vegetarian duplicate diets [30].

Market basket surveys are where shoppers collect samples that reflect the commodities widely consumed by a population but reflect a spot check rather than a robust time-averaged estimate of intake [31–33]. Food collected as part of a market basket survey is not necessarily cooked and prepared as for human consumption and so care must be taken if using data for exposure assessments. Whilst less robust, they are also less expensive and much quicker to arrange and conduct.

When planning a survey, consideration should be given to the reasons behind the survey. The questions to ask and points listed below focus on surveys conducted in order to conduct consumer exposure assessments and dietary intake calculations as listed below. Different considerations may be useful for environmental risk assessment and environmental quality monitoring [34].

Objectives

- Why is the survey being conducted?
- If the survey contains quantitative measurements, what statistics can be generated from the data?
- Consider how many samples or measurements will be needed to confirm or detect differences (e.g. time trends, geographical locations, food and product types, brands, population groups and subgroups, e.g. children, vegetarians, etc.) and ensure that subpopulations are assigned large enough sample sizes to detect any differences that may be of interest or importance.

Sample Units

- At what level should the sample be taken?
- Should samples be combined into a composite for analysis? For example, an estimate of exposure to chemicals in milk can be obtained from taking packs of milk from different supermarkets and other retail outlets and combining to reflect market share. Results from this composite would reflect average concentrations, and exposure can be calculated by using estimates of consumption (typically average and high (97.5%ile). However, if differences between milk produced at different farms are to be measured, then sample units may be taken from the milk bulk tanks from the farms and analysed individually. If differences between milk from individual cows are important, then samples need to be taken on this basis.
- Is the compound(s) being surveyed likely to be distributed heterogeneously?
- Is any information available on its likely distribution within a single sample, between samples of the same batch or between batches?
- Does the sample size or number of replicates for each brand need to reflect this potential variability, or does a large sample need to be taken and blended prior to analysis?

  Sample size: It is important to decide how many sample units need to be selected.
- Are usable results relating to parts of the overall sample needed as well as overall results?

- For example, are results needed on a regional basis or just a national basis; are results needed for several different types of food?
- What size of sample is required, e.g. weight/volume/number of individual units?

Geographical Coverage
- A decision on geographic coverage is required for the survey to ensure that it is adequately representative for the intended use of the data.
- A characteristic may be temporally variable.
- Statistical advice may be needed with respect to the sampling period required so that the results are not affected by seasonal or short-term phenomena.
- Consideration should be given to whether a food may be imported or home/locally produced.
- Is there regional variability between products?
- In certain circumstances, it may be acceptable only to sample in some areas or to adjust sampling by region to reflect differences in regional consumption habits.
- Will local practices need to be taken into account?
- Local knowledge can often provide useful advice at the planning stage.

Choice of Sampling Locations
- The range of retailers to be covered needs consideration.
- In retail surveys, samples should as far as possible be selected from a range of major and smaller retail outlets as well as independent retail outlets and should be chosen and weighted to reflect market share, unless weighting is needed to ensure a statistically adequate number of minority categories are included.

Timing
- When is the sample to be taken?
- Is the product only available at certain times of the year?
- Do products tend to originate from different places at different times of the year?
- The level of analytes found may vary with the season.

Origin of Samples
- Will imported food be included?
- If so, when and where will samples be taken?
- Consignments may enter a given country at different places and come from different countries at different times of the year or may cease at certain times of the year.

Previous or Existing Knowledge
- Make use of available information, for example, market share data, pilot study, archived survey data and scientific literature; a basis for sampling can be taken from existing data sets such as lists of food retailers held by local authorities.

Surveys may be carried out by regulatory authorities, the food industry or others, such as consumer organisations, pressure groups or academics who may be

interested in chemicals in food with a potential impact on health (food safety) or chemicals that would not normally be expected to be found although they have no known health impact (food quality). For industry, surveys of products manufactured can demonstrate due diligence in their production methods and show that every precaution is taken to ensure that products are safe and of good quality.

Surveys Can
- Help to protect and inform consumers
- Judge the effectiveness of regulation and/or production methods
- Monitor trends and to assess risks by:

  i. Protecting consumer safety (in this case, with respect to exposure to dioxins and PCBs from the diet).
  ii. Allowing consumers to make informed choices (from an ethical, environmental or health perspective).
  iii. Informing authorities in respect of the need for policy position or regulation.
  iv. Assessing the effectiveness of current legislation, codes of practice, etc., as implemented.
  v. Monitoring trends, both in terms of concentrations and geographical location and over time.
  vi. Enabling consumer exposure assessments and dietary intake calculations to be made. Such exposure assessments, when robust, form an integral part of the risk assessment process.

It is important that surveys for estimating population exposures are conducted using randomly taken samples. With budgetary restraints, there is a tendency to combine surveys with a risk-based sampling programme, for example, to check around industries known to have the potential to release dioxins or PCBs into the environment or other potential 'hot spots'. However, it should be noted that data generated from such weighted surveys may result in skewed estimates of population exposure.

3.5 Analytical Measurement Uncertainty

Uncertainties in toxicology and in particular TEFs were discussed earlier in this Chapter. Estimation of measurement uncertainty is an important part of quality control and a requirement of ISO 17025 accreditation. But there are a wide variety of approaches used in the analytical community, some based on a 'bottom-up' approach and others on a 'top-down' approach [35]. The bottom-up approach involves summing the individual errors on the different processes used in making the analytical measurement, whereas the top-down approach estimates uncertainty based on quality control data and method performance. Measurement uncertainty will be greater at lower concentrations; i.e. as the limit of quantification (LOQ) for

the method is approached. For dioxin analysis, the measurement uncertainty needs to be calculated for individual congeners and for the TEQ where this is reported. It is possible to check that estimates of uncertainty are realistic using statistical methods, namely, the Zeta score (ζ) [36].

3.6 Comparison or Compilation of Data from Different Sources

When examining data reported from a wide range of sources, it is important to establish consistent quality criteria before comparisons can be made. An example where this was important was in the EFSA report [37] that compared data on dioxins and PCBs collected from across the European Union. To ensure that only valid results were used in data analysis, EFSA evaluated the 26,600 sets of individual sample results. Data sets including results for only non-dioxin-like PCBs were deferred to a future analysis to be covered in a separate report. A list of validation steps was applied to the remaining 13,854 data sets: samples with a number of relevant fields left blank, samples where only the total $TEQ_{(WHO98)}$ was reported without individual values at the congener level or samples with other inconsistencies in the way results were reported making interpretation difficult were excluded from the data set. Only samples with complete information for the 17 congeners of dioxins and furans and for the 12 congeners of dioxin-like PCBs were retained for statistical analyses. This included an initial 6,616 samples. An imputation technique was adopted to treat missing values. An exploratory analysis of the toxic equivalent sum of dioxin, furan and dioxin-like PCB congeners showed that, overall, more than 85% of the sum was determined by five congeners (i.e. 2,3,4,7,8-PeCDF; 1,2,3,7,8-PeCDD; 2,3,7,8-TCDD; 2,3,7,8-TCDF; PCB-126). At the congener level, the LOQ values of samples for which at least the five aforementioned congeners were present were imputed using country- and food-/feed-specific LOQ median values ('cleaned number'). As a last quality control check, lower- and upper-bound estimates were compared. Lower- and upper-bound contamination values were determined by setting to zero and LOQ, respectively; congener-specific analytical results were reported to be below the LOQ. In accordance with Commission Regulation (EC) No. 1883/2006, samples were excluded when the percentage difference between lower-bound and upper-bound estimates of total dioxin levels was greater than predefined threshold values. Specifically, thresholds were set to 60% for contamination in the range of 0.2–0.4 pg $TEQ_{(WHO98)}$/g, to 50% in the range of 0.4–0.8 pg $TEQ_{(WHO98)}$/g, and to 30% for contamination levels greater than 0.8 pg $TEQ_{(WHO98)}$/g. After these exclusions, the 'final' database included 210,830 results covering 7,270 samples from 19 member states, Iceland and Norway. It is noteworthy that using the imputation technique increases the sample numbers by only 10% (from 6,616 to 7,270) and that the final 7,270 samples represented only 27% of the 26,600 sets of sample results submitted.

This highlights the complexity and the continuing difficulty of analysing and reporting results for dioxins.

3.7 Biomarkers

There is another approach to estimating dietary intake by using biomarkers. This is where the compounds of interest are monitored in some form of biological tissue or fluids taken from the exposed population. This can include urine, where typically a collection is made over a given time period, and, assuming the compound or a known metabolite is excreted at a concentration that can relate to intake (either quantitatively or by factoring in a known half-life, etc.), then exposure can be estimated. This approach was for example used in a study to monitor exposure to phthalates using urinary biomarkers [38] but is less suitable for lipophilic compounds such as dioxins and PCBs. For such lipophilic compounds with long body half-lives, exposure can be estimated from the measurement of blood or human milk samples and also normalised on lipid content [39].

Since dioxins and PCBs have such a long half-life (several years, see earlier), adipose samples taken by biopsy have also been used in contamination incidents to establish whether or not animals have been exposed and also to monitor body burden (and thus levels in meat) as the animals grow and time from exposure increases. A small section of fat from the rump of cattle taken under local anaesthetic has been the tissue typically used for this procedure.

3.8 Risk Assessment Following a Contamination Incident

A food incident may be classified using a combination of the severity of the incident and the complexity of the investigation [40]. Factors contributing to 'severity' include the extent of actual or expected health effects, number of consumers affected and perceived risk by the media. 'Complexity' can be increased when a large number of reports are received, when there is a large number of products and/or locations involved and when there are a large number of agencies involved. Traceability of food products is an important consideration and any risk assessment for a particular food incident will need to cover aspects relating to public health issues.

Following a contamination incident, it is often important to be able to conduct a risk assessment often with little time before making important decisions about matters such as product withdrawal, often with limited data. Under such circumstances, it is usually not feasible to initiate food surveys and so on in order to make robust dietary intake and exposure estimates. However, it can be concluded that data obtained from such surveys as described above plays an essential role in order to establish background or normal levels. Values found during an incident can be

quickly compared to such reference estimates to gauge the extent of the incident. Once an incident has been uncovered (if it is uncovered – there is a strong possibility that food incidents are not uncovered where regulatory food control is scarce), it is also important to assess how long the contamination has been happening. For example, following the discovery of the dioxins contamination incident in Ireland at the end of 2008, EFSA published an assessment of risk within 2 days [41]. It considered the maximum concentrations that had been found to date during the incident and compared this with the body burden and TWI. This risk assessment included the maximum time that the incident could have been in existence. Exposure scenarios were calculated for three levels of exposure up to the maximum concentrations found and for assuming that 100%, 10% and 1% of contaminated meat was consumed. In the most extreme case, assuming that 100% contaminated meat was consumed for the entire 90-day period of the incident and that all of this meat was at the highest concentration measured resulted in a reduction of the uncertainty factor associated with the TWI. Given that the TWI had a built-in uncertainty factor of 10 and given the chronic nature of effects associated with exposure to dioxins, the conclusion was that such exposure would reduce protection but not necessarily lead to adverse health effects.

A variety of options are available for risk management depending on the scale of the incident. These include communication or making alerts or information notices often through formal systems such as the Rapid Alert System for Food and Feed (RASFF) in the EU. Authorities may publish advice and guidance to consumers, such as that issued to consumers to limit consumption of oily fish sourced from the Baltic Sea because of contamination with dioxins and PCBs. Voluntary restrictions may come into operation such as complying with a period before livestock can be slaughtered for consumption. Finally, statutory restrictions may be imposed to restrict the sale or movement of agricultural produce.

3.9 Risk Assessment for Point Sources

A similar bespoke exercise (although not always necessarily carried out in such haste) may be performed for point sources of contamination. These sources may relate to food produced around an inefficient municipal or chemical waste incinerator or other combustion source. Alternatively, it could for example relate to food produced on the flood plain of a contaminated river where some of the contaminant load may be transferred to the food produced on such land.

3.10 Environmental Risk Assessment

This chapter has focussed on the risk assessment of dioxins and PCBs relating to dietary consumption since this is by far the greatest source of exposure for most

people. But dioxins, PCBs and other persistent organic pollutants can be discharged into waterways, e.g. from sewage effluent, where they can have toxic effects on fish and aquatic wildlife. There is also often a downstream intake of water which after treatment can be used as drinking water for humans. The majority of dioxins and PCBs in river water is usually bound to the sediment or suspended particles with a high carbon content. As a result of the fact that these compounds are known to bioaccumulate in fish, especially fatty fish, many surveys have been conducted on both farmed and wild fish. Dioxins and PCBs may contribute to endocrine effects seen in fish, although it is known that other chemicals may also have this kind of action.

Surveys conducted on fish are often designed to help with environmental risk assessment, and often species of fish and geographical location of sampling sites are not chosen with a direct objective to help with dietary exposure estimates – for example, species of fish widely consumed is not an important consideration in designing the surveys. Also, the monitoring of a bioindicator such as VTG measures total biological effect rather than concentrations of individual chemicals. This may be more relevant when looking at total capacity for endocrine disruption but has the disadvantage that other possible more significant toxic effects (e.g. dioxins) will be missed and it will not be known which classes of compounds are causing the problem. Other effect-based bioassays are also available, e.g. the YES yeast-based oestrogen assay and the CALUX ER assay [42]. Whilst all such assays have the advantage of covering a range of chemicals with similar modes of action and are usually rapid and relatively easy to implement, they all have similar drawbacks to those mentioned above for VTG monitoring.

Many of the aspects relating to the surveillance of foods for specific classes of endocrine-disrupting chemicals are common to factors relating to many other classes of chemicals in foods. Some of these other classes of chemicals may be present in foods either as natural toxins, or as a result of contamination, as additives or as residues of compounds applied during production.

4 Conclusion

Dioxins and PCBs are ubiquitous contaminants and we are all exposed to these chemicals primarily through our diet. Due to the highly toxic nature of these chemicals, balanced by the costs of reducing or removing these contaminants from human food, it is important that robust methods of risk assessment are available. Furthermore, dioxins and PCBs regularly feature as contaminants responsible for incidents, and it is important to have tools available for the management of such a crisis. This chapter describes the processes that are currently in place and represent the best practice.

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DDT and Metabolites

S. Mirmigkou and J. de Boer

Abstract Dichlorodiphenyltrichloroethane (DDT) is a well-known insecticide that was introduced and widely used during World War II. In total more than 4.5 million tonnes DDT have been produced. Although its use and production stopped worldwide during the 1970s, it was reintroduced in the 2000s as a malaria vector control by the World Health Organization (WHO). DDT is toxic to animals and humans. Its main characteristics are its persistence, lipophilicity, and bioaccumulative potential. DDT and its metabolites are normally determined in organisms, sediments, or soil by gas chromatography combined with either electron capture detection or, preferably, mass spectrometry. Many interlaboratory studies have been carried out on the analysis of DDT and its metabolites, and certified reference materials are now available. DDT and its metabolites have been found in air, water, sediment, and biota from all over the world. As a consequence of global fractionation and cold condensation, DDT and its metabolites accumulate in the Arctic. Since the reintroduction of DDT against malaria in Africa in 2005, monitoring of DDT compounds is again highly relevant to detect changes in environmental levels of DDT. Such monitoring is particularly needed in Africa. The first indications show an increase of DDT in humans and wildlife.

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Contents

1 Introduction

1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) was one of the first synthetic pesticides used. It was first synthesized by an Austrian chemist, Othmar Zeidler, in 1874 [1], but its insecticidal properties were not discovered until 1939, by Paul Muller, a Swiss entomologist, who won the Nobel Prize for this discovery [2]. Its first use as an insecticide was during World War II for the protection of the Allied forces from diseases such as typhus and malaria, which were spread by mosquitoes and lice to humans [2, 3]. This breakthrough, in addition to its effectiveness and low price, led to its commercialization and use for agricultural purposes [1, 2]. One of its great successes was the elimination of malaria from Europe and the USA [1].

According to the World Health Organization (WHO) specifications for public health pesticides, technical DDT is a white- or cream-colored powder consisting of DDT and manufacturing impurities. Its mixture consists of 80% *p*,*p*′-DDT and 20% *o*,*p*′-DDT [4].

Dicofol (Fig. 1) is a widely used pesticide, synthesized from DDT. It contains DDT as impurity which must not be above 0.1% according to European legislation [5].

The mass production of DDT started in 1944, and only that year, in the USA alone, 4,366 t were produced, reaching its peak in 1963 (81,154 t) because of the export market. According to the Organization for Economic Cooperation and

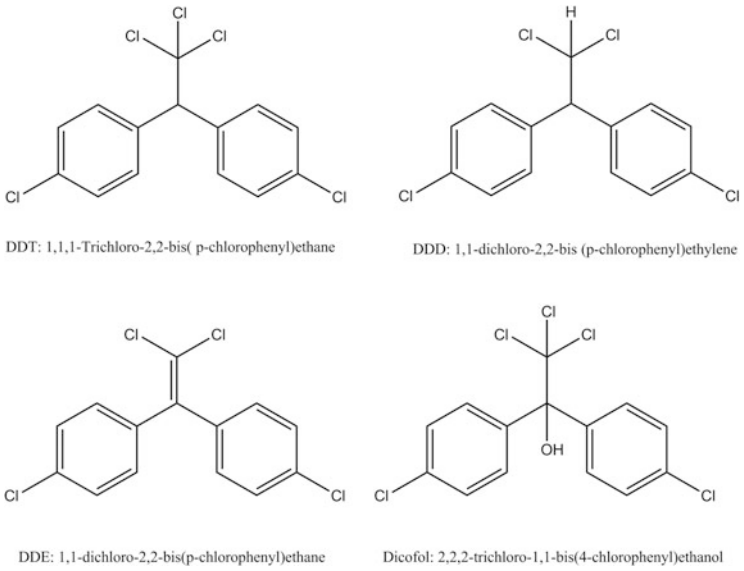

Fig. 1 DDT and its metabolites. *p*,*p*′- DDT, 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl)ethane; *p*,*p*′-DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; *p*,*p*′-DDD, 1,1-dichloro-2,2-bis (*p*-chlorophenyl)ethylene; dicofol, 2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol

Development (OECD), the worldwide annual production of DDT in 1974 was 60,000 t [6]. In the former Soviet Union, the mass production of DDT began in 1946 in Moscow, and during the 1950s–1970s, 20,000 t per year were produced. Despite its ban, the production of DDT still continued in the former USSR, and in 1986 10,000 t per year were recorded [7]. According to Li and Macdonald [8], the total worldwide production of DDT from the 1940s until now is estimated to be 4.5 million tonnes, which is almost 3.5 times higher than the estimated total PCB production, according to Breivik et al. [9, 10].

Despite its advantages, DDT was banned in most developed countries during the 1970s. This was due to its harmful effects on the environment and wildlife [11]. Properties such as chemical stability, lipophilicity, and bioaccumulation in the food chain caused a rising concern on its negative effect on humans and wildlife. These properties were also characteristic for 1,1-dichloro-2,2-bis (*p*-chlorophenyl)ethylene (DDE), one of DDT's metabolites [1].

Rachel Carson's book *Silent Spring*, in which the author outlined the global character of the DDT problem, had a great impact on the environmental movement and initiated changes in laws that protected the environment [11]. By the mid-1950s, several studies showed that DDT and its metabolites had adverse effects on the reproduction of many animals. For example, thinning of eggshells due to hormonal effects and changes in calcium metabolism by DDT [1] resulted in the potential extinction of the bald eagle, as nesting females were accidentally crushing their eggs [12]. High mortality in fish was also observed, such as young salmon and trout,

species that feed on small insects [11]. Also, a mass decline in seal population was observed with their population dropping from 3,000 to 500 within two decades, due to consumption of polluted fish [13]. The wide use of DDT resulted not only in the eradication of malicious pests but also in the death of beneficial insects. As a result, populations of previously minor insects arouse [2].

In January 1970, Sweden was the first country to ban the use of DDT due to its effect on the environment, and in the same year, the former USSR prohibited DDT for agricultural purposes. Despite its ban, DDT production and use continued illegally at many places until 1986. In the early 1970s, the USA banned the use of DDT apart from emergency public health use, and later in 1970, DDT was also banned in Norway as a general pesticide.

Now, many years after its ban, DDT is recommended again by the WHO (World Health Organization) as a malaria vector control in many African countries. As a result, the use and production of DDT is continued in countries such as Mexico, India, and several countries in Africa (e.g., Ethiopia, Mozambique, Zambia, Zimbabwe, South Africa), with India being the largest producer of DDT (4,100–4,500 t for the period 2003–2007) [14, 15]. However, until now and in spite of substantial investments in research, no other alternatives to DDT have been found with the same efficiency.

2 Toxicology

2.1 Human Toxicity

Not many cases of direct human poisoning have been reported, but there is a high concern about the chronic effects of DDT and its metabolites [2]. As a result, many studies have been conducted on the association of DDT with illnesses such as leukemia, brain cancer, prostate cancer, liver cancer, diabetes, and breast cancer [1]. The negative impact on humans of DDT and its metabolites is a rather controversial subject.

Everett et al. [16] reported an association between diabetes and DDT, not only with diagnosed diabetes but also with total diabetes [16].

In vitro studies have shown that DDT shows estrogenic activity in humans, while DDE acts as an androgen antagonist. Some of these studies suggest that DDT is also responsible for the decrease of semen quality [17–19]. Due to their lipophilic nature, DDT and its metabolites are found in relatively high concentrations in breast milk, leading to exposure of children when breast-feeding. This can turn out to be an important contamination factor for infants, especially because earlier studies showed that in Africa, mothers breast-feed their children for up to 2 years [14, 20]. In utero exposure to DDT, even in low concentrations, is shown to alternate thyroid hormones, decrease cognitive skills, and increase asthma in infants [21]. Many researchers also suggest that, due to its bioaccumulative properties,

DDT is also responsible for premature death of humans, infant mortality, and effects on the neurological development of infants [2, 21].

After the early 1990s, many studies have focused on the correlation between DDT and breast cancer. Many of them suggested that DDT and its metabolites act as tumor promoters. On the other hand, many reviews pointed out that no correlation between breast cancer and DDT exists [22–25]. Due to these inconsistent data, more research is still needed on this topic [1, 2].

The acceptable daily intake (ADI) of ΣDDT (which consists of *p*,*p*′-DDT, *p*, *p*′-DDE, *p*,*p*′-DDD) is 20 μg/kg/day as established by the FAO/WHO in 1984 [20]. According to the European Union pesticides database, the maximum residue level (MRL) for ΣDDT is 0.05 mg/kg, as set by the Commission Regulation (EU) No. 212/2013. The Codex Alimentarius recently set the MRL for ΣDDT in milk at 20 μg/kg [20].

The International Agency for Research on Cancer (IARC) and the US Environmental Protection Agency (EPA) have categorized DDT as a 2B classification (possibly carcinogenic to humans with sufficient evidence from animal bioassay data, with little or no human data), according to the IARC monograph volumes and EPA's carcinogen risk assessment guidelines.

2.2 Ecotoxicity

Toxic effects of DDT and its metabolites in animals include a disruption of the endocrine function causing, e.g., feminization of male embryos of many bird species and transformation of male fish embryos to females, among other non-desirable effects [2].

Recently, a correlation between DDT and the size of certain brain regions in birds, which are responsible for mating behavior and song, was reported. Especially in male songbirds, DDT and DDT metabolites reduced brain nuclei that are responsible for the reproductive success, reproductive behavior, and parental care with increasing levels of DDT. A significant reduction of brain and forebrain size was found after exposure to DDT during early development [26].

DDT has also an effect on many animals' main nervous system. Animals such as rats, guinea pigs, rabbits, and cats, treated with DDT, start to show tremor, ataxia, and finally epileptiform convulsions. Death is noticed due to respiratory failure. In many species DDT may cause arrhythmia and ultimately death due to ventricular fibrillation. The liver is also affected by DDT. Tests that were conducted in mice and rats showed that if fed for long periods with DDT (>2 mg/kg for mice and >5 mg/kg for rats), hypertrophy of the liver has been reported, the first changes being observed after 4 days of administration (exposure times not mentioned) [6].

2.3 Acute Toxicity

Acute toxicity of DDT to mammals is considered moderate. Oral LD50 of DDT in rats ranges from 113 to 118 and 150 to 300 mg/kg in mice, for oral administration in oil. It is less toxic for these animals when exposed via the skin, with an LD50 of 3,000 mg/kg in rats and 1,000–1,500 mg/kg in mice [6, 27]. It mainly affects the central nervous system and the liver. Low levels of DDT in humans may cause nausea, diarrhea, increased liver enzyme activity, and irritation of the eyes, nose, and/or throat [6].

The LD50 in rats of *p*,*p*′-DDD is 2,400 and 575 mg/kg of dicofol [6].

3 Analysis

DDT and its main metabolites can be analyzed by gas chromatography (GC) after extraction and cleanup, which should be optimized for each matrix.

3.1 Sampling, Extraction, and Cleanup

3.1.1 Air

Air samples are usually collected with denuder sampling devices, which concentrate the vapor insecticide in a single step. Materials such as polyurethane foam (PUF), Texan, or PUF/Texan are the most common absorbents for the collection and concentration of DDT [28, 29]. Solvent extraction is required before the sample is ready for GC analysis. Thermal desorption is an alternative method with which solvent extraction steps can be avoided [29]. Martin et al. [28] have developed this desorption method for the quantification of DDT in air. One important limitation of this method is the DDT degradation to DDE and DDD due to high temperatures during thermal desorption, which makes it difficult to measure the DDT degradation that may occur in the environment [28].

3.1.2 Water

The usual method for DDT extraction from water samples is solid-phase extraction (SPE). It is used as either an extraction technique or a cleanup technique [30, 31]. Zhou et al. [32] optimized this technique, using silica as an adsorbent. In another study, a one-step extraction of DDT and its metabolites using microwave-assisted headspace controlled-temperature liquid-phase micro-extraction was developed. Results from tests on various water samples showed that this is a rapid, sensitive, cost-effective, and eco-friendly preparation method for the determination of DDT

and its metabolites in environmental water samples [32]. Solid-phase micro-extraction (SPME) is also used for the extraction and cleanup of DDT and its metabolites in water samples. Its main advantages are the reduction of sample handling and the use of small amounts of solvents [31]. An interlaboratory study was carried out on the suitability of SPME as a sample preparation method for water samples in pesticide analysis. Eleven laboratories participated. The results showed coefficients of variation (RSD) from 24.7% to 73.3%, with 4 out of 12 results being outliers and two stragglers [33, 34]. Probably due to its limited reproducibility, SPME is not broadly used [31].

3.1.3 Soil, Sediment, and Biota

Soil and sediment are more complex matrices as these can contain sulfur and many other interfering compounds. Soxhlet extraction is the most common method of choice for the extraction of organochlorine pesticides (OCPs) in general [35, 36]. The use of a mixture of polar/nonpolar solvents is essential for the extraction of DDT that is adsorbed to organic carbon in the samples. Other techniques that are being used for DDT extraction from soil samples are supercritical fluid extraction (SFE); accelerated soil extraction (ASE), also known as pressurized liquid extraction (PLE); and microwave-assisted solvent extraction (MASE). Methods such as saponification and sulfuric acid treatment should not be used as degradation may readily occur. Several studies showed that *p*,*p*′-DDE degrades immediately after sulfuric acid treatment, and DDT and DDD convert to DDE after saponification [35]. Taylor et al. [37] used PLE for the determination of 13 OCPs, DDT and its metabolites included. Hexane–acetone (1:1 v/v) was considered to be the optimum solvent mixture for the extraction. The removal of sulfur from soil and sediment samples is important as sulfur appears as a broad peak in the chromatograms, thus disturbing the determination of DDT. Several methods can be used to remove sulfur from the extracts, such as copper rods, copper powder, mercury (although not environmentally friendly!), gel permeation chromatography (GPC), and $AgNO_3$-modified silica columns, with the last one being the most effective one, as it removes both elemental sulfur and organosulfur compounds [37, 38].

Extraction and cleanup techniques for most biota are comparable to those used for sediments. Instead of sulfur, fat is the most confounding factor. The solvent mixture is an essential factor to quantitatively extract the OCPs with the lipids from the sample [35]. In most cleanup methods, Florisil, alumina, and silica gel column chromatography are used, as they are suitable for the separation of fat and plant waxes from the samples [35, 39].

3.2 Gas Chromatography

The method of choice for the final chromatographic separation of DDT and its metabolites is capillary GC [32]. The most common detectors are the electron capture detector (ECD) and a number of mass spectrometers (MS) [28, 35, 36]. Most of the environmental sample extracts are complex mixtures. As a result co-elution of compounds may easily occur. Heart-cut multidimensional gas chromatography (MDGC) and comprehensive two-dimensional gas chromatography (GCxGC) are more effective in separation [40]. In GCxGC two ovens can be used, and specific software provides two-dimensional chromatograms [41, 42]. The splitless mode is used most frequently for injection in order to improve sensitivity [30, 32, 35]. Attention must be paid to the temperatures used with this technique, as high temperatures in the splitless mode may result in conversion of DDT into DDD. Due to dirty glass inserts and due to the use of glass wool in the liner conversion of DDT into DDE during GC injection may also occur. Open liners are, therefore, strongly recommended. Degradation over 10% was mentioned as a consequence of a dirty glass liner [44].

Nowadays, fused silica capillary columns are almost exclusively used for the determination of OCPs in general and DDT in particular. The length is usually 25–50 m with an internal diameter of 0.15–0.25 mm. The film thickness should be $>$0.15 mm to prevent on-column degradation. Fifty meter columns provide a better separation of the analytes but result in longer retention times, thus increasing on-column degradation [35]. Using 50 m columns, PCBs may be separated from DDT, but this needs to be validated. Alternatively, PCBs can be separated from DDT and other OCPs using silica gel column chromatography prior to the GC analysis [45]. The most common capillary column used for the detection of DDT and its metabolites is the HP5-MS column [30, 32, 46].

During GC analysis, interferences may be present. For example, toxaphene may be present in some samples. Toxaphene is a pesticide consisting of a complex mixture of chlorinated camphenes, bornanes, and bornenes, and the peaks may interfere with DDT and its metabolites, hindering their quantification. Numerous studies showed that it is very difficult to separate these compounds in a mixture, even when using different columns in GC. Toxaphene is an aliphatic mixture, which means that the response factors with ECD are much lower than those of the aromatic DDT compounds. That means that in samples from most areas, toxaphene is not a very serious interference. Attention should however be paid to samples from areas that are highly polluted with toxaphene, such as samples from the Arctic. GCxGC may also help for such samples.

The combination of GC and ECD is a valuable and relatively inexpensive method for the determination of DDT and its metabolites, as ECD has a high sensitivity for halogenated aromatic compounds such as DDT [35, 47, 48]. The so-called micro-ECDs are even more sensitive. It is a very common detector, especially for routine environmental analysis, and it is much more affordable than a mass spectrometric (MS) detector [41]. Its only drawback is its poor linearity

especially in the low pg range. This problem can be solved with multilevel calibration [35].

GC coupled to MS is another useful technique for the identification of DDT and its metabolites. The MS linearity is much better than that of ECD [35]. It is suitable for qualitative and quantitative analysis of DDT, as it has the ability to detect ultra-trace concentration levels of these compounds [32]. The only disadvantage of GC–MS is its relatively high running cost for a laboratory that carries out routine analysis in environmental samples. However, there has been a major development in MS during the last decades. Sensitivity has increased a lot, whereas prices have dropped considerably. High-resolution sector MS systems are not needed any longer to obtain enough sensitivity for many compounds including DDT. Benchtop quadrupole MS systems, time-of-flight (ToF) MS, orbitraps, and many other systems are nowadays available. These are still more expensive than ECD, but they do offer a wealth of possibilities, including a very high resolution and great sensitivity, which are all very useful for the analysis of DDT, in particular in combination with many other compounds. Another possibility is tandem mass spectrometry, which monitors specific collision-induced dissociation reactions, thus lowering the chemical background and increasing the signal to noise ratio. Also in GC–MS, DDT degradation can occur.

When using a mass spectrometry analysis, much interference from the matrix of the sample can occur. The internal standard method is the ideal one, in order to overcome such interferences. ^{13}C-labeled standards in an isotope dilution can be used for best results, as they have the same physicochemical properties and chromatographical behavior as the target compound, but different m/z ratio [49].

3.3 Interlaboratory Studies and Certified Reference Materials (CRMs)

Since the late 1970s, the analysis of DDT compounds has been evaluated in many interlaboratory tests. There are numerous organizations that conduct such tests, among which the United Nations Environment Programme (UNEP, Geneva, Switzerland); SETOC/WEPAL, Wageningen, Netherlands; and QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe), also in Wageningen, Netherlands. A recent interlaboratory study with a participation of 103 laboratories from all over the world was conducted by UNEP for the evaluation of the level of performance on persistent organic pollutant (POP) analysis. The laboratories were requested to analyze two test solutions and fish, sediment, fly ash, and human milk. The extraction and cleanup protocol, spiking schemes, standards, and internal QA/QC were left to the choice of the participating laboratories, but validation of their methods was very much emphasized. The only mandatory parameter was the use of capillary gas chromatography columns, to

Table 1 Assigned values, relative standard deviations (RSDs), and number of submitted results (*n*) in the first UNEP interlaboratory study on POPs [43]

	Standard solution				Sediment			
	n/n^a	Assigned value (ng/mL)	Model RSD (%)	Inclusion rate[b] (%)	n/n^a	Assigned value (µg/kg)	Model RSD (%)	Inclusion rate[b] (%)
p,*p*′-DDT	46/0	36	18	66	32/1	18.1	48	66
p,*p*′-DDE	52/0	35	15	69	37/1	15.4	44	74
	Fish				Milk			
	n/n^a	Assigned value (µg/kg)	Model RSD (%)	Inclusion rate[b] (%)	n/n^a	Assigned value (ng/kg)	Model RSD (%)	Inclusion rate[b] (%)
p,*p*′-DDT	28/0	NA	107	79	13/2	0.003	40	56
p,*p*′-DDE	34/0	8.88	80	73	20/0	NA	64	72

NA due to the limited quality of the data, no assigned value could be designed
[a]Number of submitted results not including values below limit of detection (LOD) or limit of quantification (LOQ) (first number) and (second number) the number values below LOD or LOQ
[b]percentage of laboratories on which assigned value is based

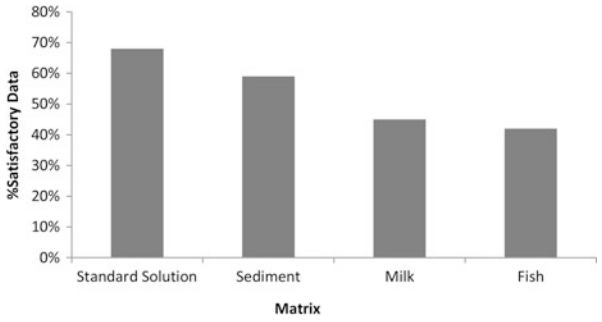

Fig. 2 Percentage of laboratories with satisfactory z-scores (i.e., $z \pm 2$) in the test solution, sediment, milk, and fish for ∑DDT [43]

achieve the right separation for the determination of the analytes. Table 1 shows the results of this study.

The percentage of satisfactory results is given in Fig. 2. The best z-scores were obtained for the standard solution. From this study it became clear that not all laboratories perform well in the analysis of all compounds, but many were specialized in specific categories [43]. It should be noted that many of the participants were from developing countries who participated in such a test for the first time, sometimes after having received only a basic training. The QUASIMEME proficiency tests show a better comparability, caused by the participation of more experienced laboratories. In general GC–MS results are better than those from ECD, due to the higher resolution of GC-MS and lower specificity of ECD [43].

The concern on the presence of DDT and OCPs in general and their effect on the environment has led to an increasing demand for certified reference materials

(CRMs) or standard reference materials (SRMs). However, there are not many CRMs/SRMs available for these compounds, as their production and certification is a relatively expensive and slow process. The European Institute for Reference Materials and Measurements (IRMM, Geel, Belgium), the US National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), and the Canadian National Research Council (NRC, Ottawa, Canada) are the three main producers in the world of CRMs. For OCPs, eight CRMs are available in biota: two cod liver oils, four mussel tissues, whale blubber, and a sea plant homogenate, which are shown in Table 2 [50].

4 Environmental Occurrence

4.1 Air

The most common DDT disposal into the atmosphere is by agricultural spray drift, post-application vitalization, and wind erosion [51]. A study conducted in China showed that DDT and DDT metabolite concentrations were higher in urban areas than in nonurban areas [47]. The concentrations were measured in the gaseous and particular phase and showed that the DDT compounds mainly occur in the gaseous phase (Fig. 3) [47].

Seasonal influences play a role in the DDT monitoring in air, as DDT levels are usually higher in summer than winter (Fig. 4) [47, 51].

The Global Monitoring Plan (GMP) for persistent organic pollutants (POPs) was built with the support of the United Nations Environment Programme (UNEP)/ Global Environmental Facility (GEF) involving countries from West and East Africa, the Caribbean, Latin America, and the South Pacific. Air was sampled by the use of PUF disks for passive sampling, and DDT and its metabolites were often the compounds occurring in the highest concentrations (Table 3) [46].

4.2 Water

DDT concentrations in water are very low, as these compounds are highly hydrophobic. Much higher levels are found in particulate matter than in the dissolved phase [48, 49]. The most common sources of DDT in water are from former use in agriculture, atmospheric deposition, and diffuse pollution from erosion processes [49]. *o*,*p*′-DDT is more persistent than *p*,*p*′-DDT and found in higher concentrations in water samples [48, 49]. Iwata et al. [52] performed an impressive cruise during which they sampled air and analyzed ocean water for several organochlorine contaminants. They found total DDT concentrations to be much lower levels than HCH levels, obviously also related to the higher water solubility of HCHs.

Table 2 RMs in biota [50]

	SRM 1974a	SRM 1588a	SRM 1945	SRM 2974	SRM 2977	SRM 2978	140/OC	BCR 598
Organization	SRM NIST	SRM NIST	SRM NIST	SRM NIST	SRM NIST	SRM NIST	IAEA	BCR
Country of origin	USA	USA	USA	USA	USA	USA	Monaco	EC
Matrix	Mussel tissue	Cod liver oil	Whale blubber	Mussel tissue	Mussel tissue	Mussel tissue	Focus (sea plant homogenate)	Cod liver oil
Units	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg
As	Dry weight	Wet weight	Wet weight	Dry weight	Dry weight	Dry weight	Dry weight	Wet weight
[±] expressed as	±95% CI	±95% CI	±95% CI	±95% CI	±95% CI	±95% CI	±95% CI	±95% CI
Units of issue	3 × 15 g	5 × 1.2 ml/ ampoule	Set 2.15 g/ ampoule	8 g	10 g	10 g	30 g	5 g
Form	Frozen	Oil	Frozen	Freeze-dried	Freeze-dried	Freeze-dried	Freeze-dried	Oil
2,4′-DDE	5.26 ± 0.27*	22.0 ± 1.0	12.28 ± 0.87	5.26 ± 2.8*		4.41 ± 0.56		
4,4′-DDE	51.2 ± 505	651 ± 11	445 ± 37	51.2 ± 5.7	12.5 ± 1.63	37.5 ± 1.5	1.2 (0.86–1.6)	610 ± 40
2,4′-DDD	13.7 ± 2.8*	36.3 ± 1.4	18.1 ± 2.8	13.7 ± 2.8*	3.32 ± 0.29	10.5 ± 1.0		30 ± 4
4,4′-DDD	43.0 ± 6.3	254 ± 11	133 ± 10	43 ± 6.4	4.30 ± 0.38	38.8 ± 2.3	0.7 (0.61–0.90)	400 ± 30
2,4′-DDT	8.5 ± 4.9*	156.0 ± 4.4	106 ± 14	8.5 ± 1.9*		9.2 ± 1.6		
4,4′-DDT	3.91 ± 0.59	524 ± 12	245 ± 15	3.91 ± 0.60	1.28 ± 0.18	2.84 ± 0.28	2.2 (1.4–3.6)	179 ± 18

For non-IAEA materials, values indicated by an asterisk (*) are noncertified. For IAEA materials, values indicated by an asterisk are classified as information values; all other values are classified as recommended

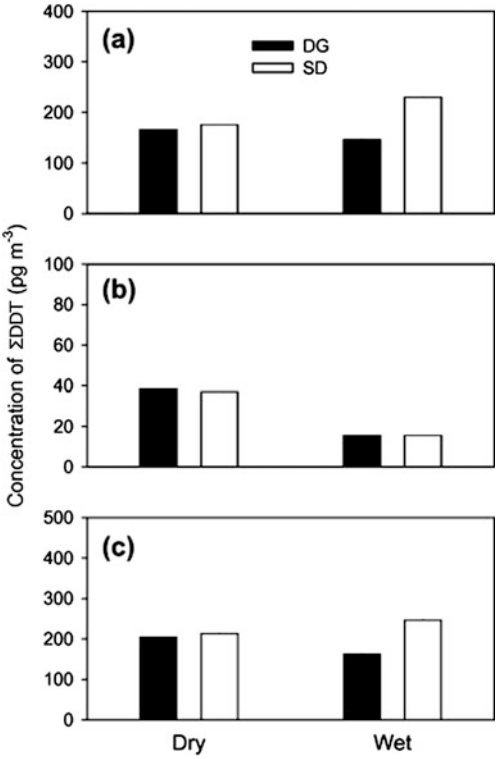

Fig. 3 Average concentrations of DDTs (sum of *o*,*p*′- and *p*,*p*′-DDT, DDE, and DDD) in (**a**) vapor phase, (**b**) particulate phase, and (**c**) air samples (gaseous + particulate phases) from Dongguan (DG) and Shunde (SD) obtained in dry and wet weather seasons [47]

Furthermore, they found DDT concentrations much higher in tropical waters and air from southern latitudes compared to northern areas.

4.3 Sediments and Suspended Particular Matter

Suspended particular matter and bottom sediments are the main reservoir of DDT in marine, lake, and river ecosystems [48, 49, 51]. The main inputs of DDT in sediments are agricultural runoff, sewage discharge, atmosphere disposition, and wastewater. Pham et al. [49] conducted a study on suspended sediment in the St. Lawrence River in Canada. ΣDDT concentration in 93 samples was found from non-detectable to 0.34 μg/g, with the most contaminated sediment coming from the Great Lakes and the Ottawa River. Higher concentrations were found during spring probably due to surface runoff and erosion [49]. Another study conducted in Greenland showed that concentrations of DDT in sediment from the

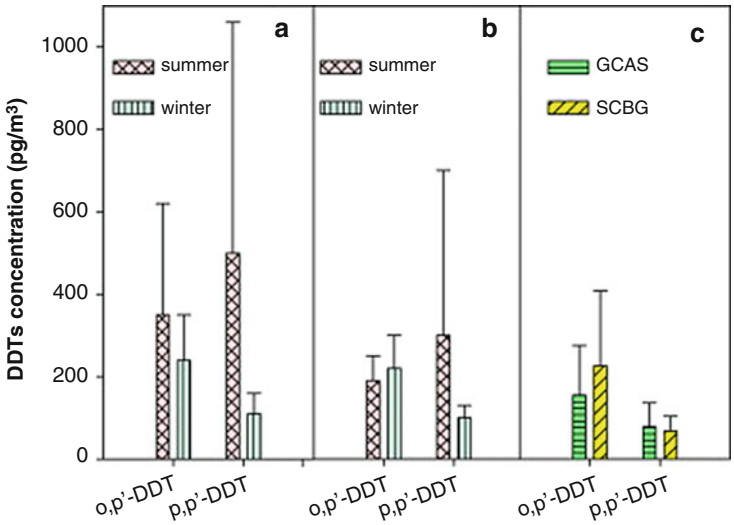

Fig. 4 Levels of DDTs (mean ± standard deviation) in air of (**a**) the Pearl River Delta (**b**) Hong Kong and (**c**) Guangzhou at an urban site, Guangzhou Branch, Chinese Academy of Science (GCAS), and a suburban site, South China Botanical Garden (SCBG) [51]

Greenland coast are comparable with those from other Arctic regions. On the other hand, data from European estuaries for DDTs were much higher than those found in Greenland [53]. Kelderman et al. [54] studied the pollution of Delft canal sediments and found that DDT and its metabolites were the dominant contaminants. Although DDT was banned in the Netherlands since 1973, these high concentrations of DDT are presumed to be due to their strong persistence in the environment [54].

4.4 Soil

In a study that took place in Guangzhou, China, surface and subsurface vegetable soil samples showed a large variation in DDT levels (3.6–831 ng/g) [51].

Depending on the conditions of the soil, two pathways of degradation may occur: aerobic (*p*,*p*′-DDE as a degradation product) and anaerobic (*p*,*p*′-DDD as a degradation product) [4]. A study conducted by Wang [55] showed that DDT concentrations in soil are dependent of its agricultural use. After growing specific plant species in DDT-contaminated soil, the total amount of DDT and DDE in soil had decreased, and this decrease was equal to the total amount of DDT and DDE measured in the plants [55]. In this way, phytoremediation of DDT-contaminated soil can take place. Another important factor for DDT degradation in soil is the pH value. There seems to be a strong correlation between pH and concentrations of DDT and its metabolites in soil. A study showed that at pH < 8.0, DDT metabolites were present in higher concentrations than at pH > 8.0. This may be caused by the

Table 3 DDT and its metabolite concentrations in selected first exposure PUF samples in ng/PUF measured by UNEP expert laboratory (IVM)

Country code	MLI	SEN	GHA	NIG	TGO	COD	ETH	KEN	ZMB	MUS	UGA	FJI	KIR	WSM	TUV	SLB	BRB
o,p′-DDE	56	9.5	<0.6	<1.3	<1.3	1.9[a]	4.5[a]	1.8[a]	14	<1.6	1.3[a]	6.4	0.32[a]	52[a]	<0.29	1.4	<0.06
p,p′-DDE	400	60	11	2.0[a]	5.7	46	26	12	140	5.7	18	59	7.7	230	1.2	81	0.13[a]
o,p′-DDD	26	2.8[a]	1.3[a]	1.3[a]	<1.3	<1.5	<1.6	<1.6	2.8[a]	<1.6	1.4[a]	<1.4	1.5[a]	5	4.4[a]	2.2[a]	<0.08
p,p′-DDD	27	3.2[a]	3	<1.3	<1.3	<1.5	2.5[a]	<1.6	6.6	<1.6	1.1	26	6.2	4.2	<1.5	9.6	<0.14
o,p′-DDT	34	9.7	3.7	<1.3	<1.3	10	29	3.1	180	6.9	<1.1	63	6.7[a]	53	19	36	<0.14
p,p′-DDT	70	27	8.5	1.5	6.4	27	60	8.2	310	4.8	8.8	180	15	47	5.8[a]	580	0.31

Country codes: MLI Mali, SEN Senegal, GHA Ghana, NIG Nigeria, TGO Togo, COD Congo, ETH Ethiopia, KEN Kenya, ZMB Zambia, MUS Mauritius, UGA Uganda, FJI Fiji, KIR Kiribati, WSM Samoa, TUV Tuvalu, SLB Solomon Islands, BRB Barbados [46]

[a]Indicates value between limit of detection and limit of quantification

change of the humic acid structure from fibrous to smaller sheetlike structure, at pH > 7.0 [4].

4.5 Aquatic Organisms

Fishes are generally used for environmental monitoring, as the contaminant loading in fish can reflect the state of their ecosystem [51]. A study conducted in Baltic herring and pike showed large variations in DDT levels (ND–10 mg/kg), depending on the differences in the fat content of the fish. Also, DDT levels vary between tissues of muscle and some organs. For example, in the same study, kidney of pike appeared to be a possible storage site [56]. The fat content of the various tissues is determining the final concentrations of DDT. For example, in cod muscle tissue with a fat content of ca. 1%, the DDT concentrations are much lower than in cod liver, which has an average fat content of 50% [57]. On the contrary, in eel, the DDT concentrations are much higher in the muscle tissue, which has a fat content of 5–40%, compared to those in the liver of the eel with a fat content of <5% [58]. DDT levels in farmed fish (freshwater and seawater) vary with the contamination of the DDT level in their feed [51].

Marine shellfish, especially mussels, are often used as local indicators of DDT contamination, as these species are highly bioaccumulative and do not migrate. In a study on OCPs in mussels and oysters collected from the east coast of Thailand, DDT was the contaminant with the highest concentrations in both organisms. This study also concluded that there is a decreasing trend of DDT compounds in mussels since 1979 [59].

5 Time Trends

Several publications have appeared on temporal trends of DDT and its metabolites. A study on DDT and its metabolites in cod liver oil from the Baltic Sea during the period 1971–1989 showed that DDT concentrations were rapidly declining from 1974 onwards. ΣDDT ranged from 0.4 to 25 μg/g (on a lipid weight (lw) basis). *p*, *p*′-DDE was the dominant compound followed by *p*,*p*′-DDD and *p*,*p*′-DDT [60]. The NOAA mussel watch program shows a decline of total DDT concentrations (mainly DDE) from 1,600 to 300 ng/g dw over the period 1986–2002 in Palos Verdes, CA, USA [61]. Macgregor et al. [62] reported a declining trend in eel in most, but not all, locations in Scotland. On the other hand, Suns et al. [63], after studying ΣDDT temporal trends in spottail shiners from the Great Lakes over the period 1975–1990, concluded that although there has been a decline in DDT concentrations during these years, they tend to stabilize after the 1980s, especially DDE [63]. Zhang et al. [64] reported increasing DDT levels in sediment cores from the Pearl River Delta, China. They assumed that excessive soil runoff enhanced by the large-scale land transform and regional flooding in the Pearl River Delta might

have contributed to the transport of organochlorine pesticides from soil to the sedimentary system in the early 1990s. They also found increasing DDE/DDT ratios, pointing to former rather than recent use of DDT. An atmospheric monitoring program (1993–2006) in the Arctic showed, in general, low levels of DDT-related compounds during the entire period. Although there was a reduction in concentration of these compounds in the Arctic during the 1990s, this reduction was lower during later years, and concentrations of DDT and its metabolites are almost stable now. The only exception was during the summer of 2004 when a summer forest fire occurred in Yukon, Canada. During that period, high concentrations of *p*,*p*′-DDE and *o*,*p*′-DDE were observed at two stations, probably due to biomass burning. No seasonal profiles were observed, apart for *p*,*p*′-DDE, in which concentrations were higher during the winter period [65]. Guglielmo et al. [66] studied the global environmental cycling of DDT in the 1980s for one decade. Figure 5 shows that multiple cycles are responsible for the DDT burden in soil, vegetation, ocean water, and sea ice [66]. The study concludes that after 10 years, approximately 12% of DDT would have accumulated in the Arctic. Assuming continuing low DDT emissions, Schenker et al. [67] showed in a model that concentrations will decrease by a factor of 30 in temperate regions and by a factor of 100 in the Arctic, as compared to the concentrations in the 1960s and 1970s. In the tropics, levels decrease by a factor of 5 to 10, only.

In 2002, a POP assessment in the Arctic occurred by the Arctic Monitoring and Assessment Programme (AMAP). In this assessment, temporal trends of POPs in air, water, sediment, and Arctic biota were examined. Many interesting conclusions came out of this assessment. Data of temporal trends of POPs in Arctic air were available from five air monitoring stations in the Arctic. Data was collected from 1993 to 2000. The main DDT metabolites that were found were *o*,*p*′-DDE and *p*,*p*′-DDE. Seasonal variation was present at all sites. According to Hung et al. [68], *p*,*p*′-DDE is decreasing slightly at the Alert station, while a slight increase of *o*, *p*′-DDT during the period 1993–1998 was also reported. This could be due to an unknown source causing technical DDT entering the Arctic [68].

There are a number of temporal trend data of DDT in freshwater and sediments in the Arctic. Zhulidov et al. [69] reported ΣDDT changes in water and sediments from eight Russian Arctic rivers during the period 1988–1994. Concentrations of ΣDDT declined over the years, reaching detection limits of ng/L magnitude by 1992 (Fig. 6) [69].

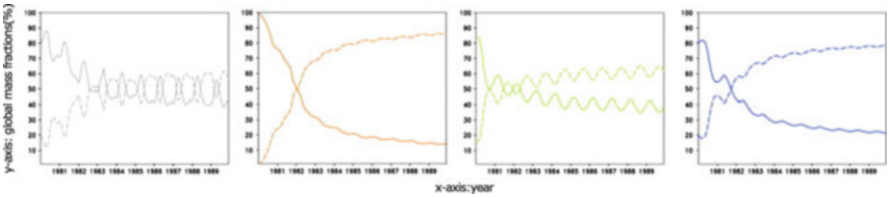

Fig. 5 DDT global mass fractions (%) in atmosphere (*gray*), soil (*orange*), vegetation (*green*), and ocean and sea ice (*blue*) in first cycle (*single hop*, *full line*) and during subsequent cycles (*multi-hop*, *dashed line*) [67]

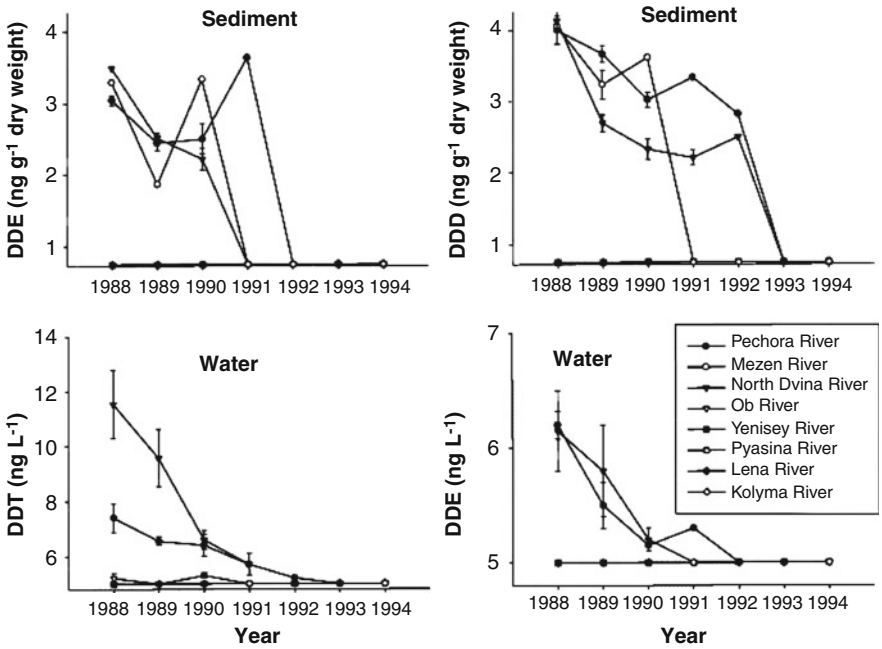

Fig. 6 Temporal changes in DDT, DDD, and DDE concentrations (mean ± S.E.) in the water and sediments of eight Russian rivers [69]

All temporal trend studies carried out in the Arctic indicate that, although there has been a substantial decrease of DDT and its metabolites in biota since the 1970s, concentrations of ΣDDT stabilized from the 1990s to the 2000s [70].

Research on POPs was not only conducted in the Arctic ecosystem but also in Antarctica. Most of the studies concluded that *p*,*p*′-DDE in particular is the most persistent organic contaminant in Antarctic biota [71–73]. Trends of DDT and its metabolites in Antarctic biota showed a decreasing pattern during the period 1980–1995, but increased again in 2000–2002 [74]. The main reasons that these compounds are found in the Arctic and the Antarctic are the global fractionation and cold condensation processes [75]. Comparing data from research in the Arctic and the Antarctic shows that concentrations of DDT found in the Arctic are much higher than those found in the Antarctic. This is mainly due to geographical features. The Antarctic region is surrounded by the Southern Ocean, which can act as a boundary for POPs. Therefore, they can reach the Antarctic only through air mass transport [74, 75]. The Southern Hemisphere contains less land mass, which is also relatively far away from the South Pole. In addition, the population density at the Southern Hemisphere is relatively low, while, on the contrary, the dense population of the Northern Hemisphere causes a higher DDT level in the Arctic [74]. The increase of DDT in Antarctica is, therefore, very interesting, as the question is if this is related to the renewed application of DDT in southern Africa. Although based on a limited data set, Alava et al. [15] reported a significant increase in DDT concentrations in

Galapagos sea lion pups between 2005 and 2008 (280–530 μg/kg lipid weight). These levels are able to affect the immune system and population dynamics during periods of nutritional stress such as El Niño events. The authors concluded that DDT and associated health risks in wildlife are generally believed to be declining, but this may no longer be the case in tropical countries where DDT is increasingly used. Lopez-Carillo et al. [76] expressed their concern in 1996 about relatively high DDT levels in Mexico, higher than in other Latin-American countries. Women living in Mexico City showed up to 0.6 mg/kg lipid weight in their breast milk. Increasing DDT trends were also suggested by Manaca et al. [77]. Examination of the distributions of DDT and its metabolites in the walls of 43 dwellings from Manhiça (Mozambique) showed median levels of 19, 130, and 23 ng/g for *o*,*p*′-DDT, *p*,*p*′-DDT, and *p*,*p*′-DDE, respectively, directly after indoor residual spraying (IRS). The concentrations of these compounds at the onset of the IRS campaign ($n = 48$) were 5.5, 47, and 2.2 ng/g, respectively. In another study, Manaca et al. [78] reported concentrations of DDT compounds in the cord blood of 214 children born between 2003 and 2006, so before IRS, in Manhiça (Mozambique) of 0.8 and 0.4 ng/ml for *p*,*p*′-DDE and *p*,*p*′-DDT, respectively, were similar to those found in western countries. However, the *p*,*p*′-DDT/*p*,*p*′-DDE ratio was high indicating an association with recent use of DDT. A significant increase in *p*,*p*′-DDT and its main metabolite, *p*,*p*′-DDE, in mother's milk samples from Manhiça was observed between 2002 (median values 2.4 and 0.9 ng/ml, respectively) and 2006 (7.3 and 2.6 ng/ml, respectively). Grimalt [79] reports a further significant increase of DDT and its metabolites in the same mother and newborn cohort in 2010. Channa et al. [17] found elevated *p*,*p*′-DDT/*p*,*p*′-DDE ratios in coastal KwaZulu-Natal, also outside the endemic malaria areas, which indicates recent ongoing and possibly illegal use, since DDT use is only allowed in designated areas. The DDT concentrations reported by Manaca et al. [77] and Grimalt [79] are above the thresholds for deleterious effects in cognitive skill observed in children at 4 years of age. This was also confirmed by Bouwman et al. [80] who reported DDT levels in 163 breast milk samples from South Africa. Mean ΣDDT levels in breast milk were 18, 11, and 9.5 mg/kg lipid weight from three DDT-sprayed villages, respectively, including the highest ΣDDT level ever reported for breast milk from South Africa (140 mg/kg lipid weight). A provisional tolerable daily intake for DDT by infants was significantly exceeded. Van Dyk et al. [81] reported elevated DDT levels in the Limpopo province in South Africa and raise concern on the potential health effects in residents living in the immediate environment following DDT IRS.

6 Conclusions

At present, DDT's use is mainly as an insecticide in African countries for the fight against malaria. However, its effect on future generations is concerning many researchers [82]. Its persistence, estrogenic activity, lipophilic nature, and ability

to bioaccumulate in higher organisms of the food chain are the main factors of concern as DDT, and its metabolites seem to be responsible for illnesses such as diabetes, asthma, and cancer. More than 75 years after its introduction, DDT and its metabolites are still present in many organisms and abiotic environmental compartments all over the world which shows the extreme persistence of this compound. As its main use is for malaria vector control, there is a need to search for alternatives of DDT, which would be less toxic to living organisms and more environmentally friendly. The two main alternatives are indoor residual spraying and insecticide-treated bed nets with pyrethroids instead of DDT [14]. The main drawback of pyrethroids is that some mosquito breeding, particularly in Africa, seems to be resistant to this particular insecticide [14]. Another alternative that has the prospect to eliminate completely malaria is malaria vaccination. Much research has been done on this matter for more than 30 years [83–85], but only recently tests and clinical trials have been conducted [84]. DDT is still present in the environment and it is unknown for how long it will exist. Most trend studies show that there has been a decreasing trend of DDT concentrations in the environment, but these concentrations seem to stabilize over the last decade. This may be caused by its reintroduction as a malaria vector control in many African countries, but this needs further evidence. There is no concrete evidence that DDT and its metabolites can be eliminated completely from the environment. Ongoing environmental monitoring is important, especially in Africa, to detect a possible increase in DDT levels in the environment, as is suggested by a study in sea lion pups from the Galapagos Archipelago [15] and several studies recently carried out in Africa [21, 77, 78, 80, 86]. The Global Monitoring Plan of the Stockholm Convention may enable such monitoring in the near future [46].

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Brominated Flame Retardants

Adrian Covaci and Govindan Malarvannan

Abstract Brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs), and tetrabromobisphenol-A (TBBP-A), have routinely been added to consumer products for several decades in an effort to reduce fire-related injury and property damage. Yet, concerns for this class of chemicals have risen because of their occurrence in the environment, wildlife, and humans. Here, we briefly present the major scientific issues related to analytical, toxicological, and environmental aspects of these BFRs and discuss data gaps. Regarding geographical or temporal trends in the environmental levels of BFRs, few general remarks may be deducted: (a) Concentrations of BFRs in general are often elevated by at least one order of magnitude in the vicinity of point sources. (b) Detection of BFRs in remote location implies long-range transport and detection in air samples. (c) In general, the continental market demands seem to be reflected in different environmental residue levels and trends.

Keywords Brominated flame retardants, Environment, e-Waste, Human exposure, Indoor, Toxicology

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Contents

1 Introduction

Flame retardants (FRs) are chemicals that are added to polymers which are used in plastics, textiles, electronic circuitry, or other materials to inhibit or retard the fire spreading [1, 2]. The different classes of FRs include inorganic materials, such as antimony oxides, aluminum and magnesium hydroxides, and borates; organic phosphate esters with or without halogens; and chlorinated and brominated organic compounds [2]. The most commonly used brominated flame retardants (BFRs) are polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), and tetrabromobisphenol-A (TBBP-A). FRs act by different mechanisms depending on the respective chemical class: Some compounds break down through endothermic processes when subjected to high temperatures (e.g., aluminum hydroxide) and thus "cool" the fire, while other compounds act as diluents of fuel or of the gas phase (calcium carbonate, carbon dioxide, or water). Chlorinated and brominated FRs under thermal degradation conditions act through another mechanism and release HCl and HBr which react with highly reactive H• and HO• radicals present in the flame resulting in the formation of inactive molecules and of Cl• or Br• radicals. The halogen radicals have a low potential to propagate the radical oxidation

reaction and therefore the flame. Despite claimed benefits for reducing fire-related injury and property damage, growing concerns for BFRs have risen because of their occurrence and persistence in the environment, biota, and humans, in a similar way to other persistent organic pollutants (POPs) [3–5].

1.1 Polybrominated Diphenyl Ethers (PBDEs)

PBDEs have a common structure of a brominated diphenyl ether molecule that may have anywhere from 1 to 10 bromine atoms attached. Depending on the location and number of bromine atoms, there are 209 possible PBDE compounds; each are termed congener and are assigned a specific number (chemical structure of PBDEs in Fig. 1; [5]). There are three major PBDE commercial formulations: penta-, octa-, and deca-BDEs, according to the number of bromine atoms in the dominating congeners. The three PBDE mixtures have different applications: (a) Penta-BDE mixture is primarily used in foams, such as seat cushions and other household upholstered furniture, in typical concentrations between 2 and 6% (w/w), as well as in rigid insulation and also used in printed circuit boards in electronics. (b) Octa-BDE was mainly used in a variety of thermoplastic resins, in particular acrylonitrile–butadiene–styrene (ABS) plastics, which can contain up to 12% by weight octa-BDE [6]. (c) Deca-BDE was used in plastics and textiles. In plastics, deca-BDE was primarily used in high-impact polystyrene (HIPS) for electrical and

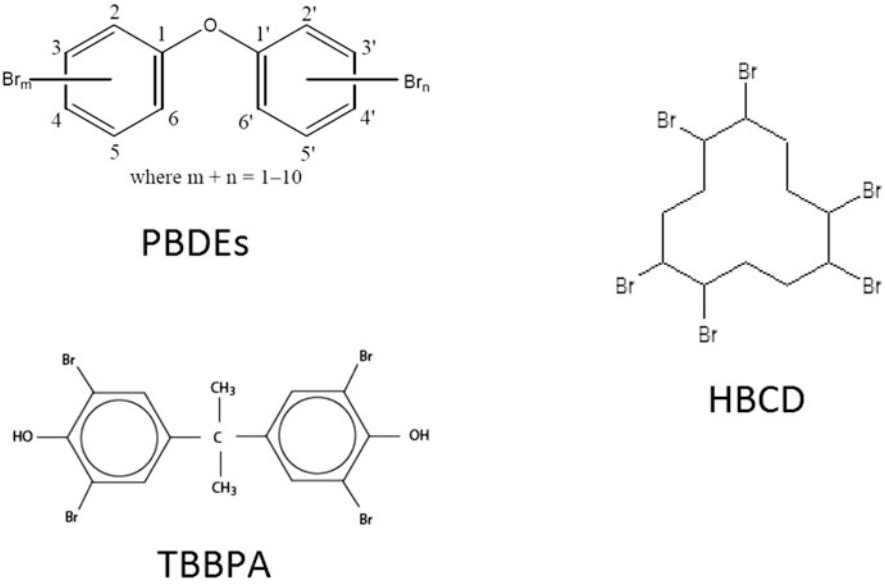

Fig. 1 Chemical structures of major brominated flame retardants: polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), and tetrabromobisphenol-A (TBBP-A)

electronic equipment in concentrations up to 20–30% (w/w) (i.e., housings of computers and TV sets), in the transportation and aeronautic sectors (i.e., planes, cars, and trucks), and in construction and building (i.e., wires and cables, pipes, and carpets). In textiles, deca-BDE was used as a fabric treatment and coating on carpets and draperies, but not in clothing.

Because of health and environmental concerns, the penta- and octa-BDE commercial mixtures have been banned or phased out of production and use in the USA and Europe, although they are still used in other parts of the world [5, 7–9]. Furthermore, the use of deca-BDE has been banned in the European Union (EU) in electrical and electronic applications since July 2008 [10], while congeners of the penta-BDE and octa-BDE have been added to the POPs list of the Stockholm Convention [11].

1.2 Hexabromocyclododecane (HBCD)

The commercial HBCD consists in a mixture of the *alpha*-, *beta*-, and *gamma*-HBCD diastereomers (HBCDs), with the *gamma*-HBCD isomer being dominant ($\geq$70%). HBCD is a high-production volume chemical (16,700 tons in 2001) and a priority pollutant of the European Chemicals Bureau (general chemical structure of HBCD in Fig. 1). It is mostly used in extruded (XPS) and expanded (EPS) polystyrene foams and as insulation material in construction industry. HBCD is highly efficient so that only low percentages (0.7% in EPS and 2.5% in XPS) are required to reach the desired flame retardancy. Other uses of HBCD are upholstered furniture, automobile interior textiles, car cushions and insulation blocks in trucks, packaging material, and electric and electronic equipment. In 2013, HBCD has been added to the POPs list of the Stockholm Convention, while in Japan it is classified as a type I monitoring substance together with PBDEs and TBBP-A. HBCDs, similarly to PBDEs, are not chemically bound to the polymers and, consequently, may escape during production, use, disposal, and recycling processes.

1.3 Tetrabromobisphenol-A (TBBP-A)

With a global production of 170,000 tons in 2004 [12], TBBP-A is the most used BFR (chemical structure of TBBP-A in Fig. 1). TBBP-A is mainly used as a reactive BFR in laminates for printed circuit boards used in electronic devices. Additionally, TBBP-A is used as an additive BFR in acrylonitrile–butadiene–styrene (ABS) polymers and an intermediate in the production of other BFRs, such as TBBP-A derivatives and brominated epoxy oligomers [13]. Following favorable risk assessments, the use of TBBP-A is not restricted in any country [12].

1.4 Novel Brominated Flame Retardants (NBFRs)

BFRs which are new to the market or newly or recently observed in the environment are referred as NBFRs [14]. The recent bans and restrictions of the use of PBDEs have paved the way for the use of NBFRs as FR replacements. The estimated total volume of NBFRs in 2009 was between 100,000 and 180,000 tons/year [15]. This high uncertainty derives from the estimates of the production volumes of 21 NBFRs. In general, halogenated FRs represent about 25% by volume of the global production of FRs with a growth of around 5% per year [16].

2 Analytical Methods

Initial analytical methods for the determination of BFRs were in most of the cases based on protocols previously established for POPs, such as polychlorinated biphenyls (PCBs). Even if BFRs have slightly different properties from PCBs, such as polarity and vapor pressure, common approaches can be still used [17–21]. A rapid growth in the number of BFR specific methods has been seen in the last decade. Due to their particular physicochemical properties, the determination of individual HBCD isomers and TBBP-A may require specific analytical approaches. Basic steps of the BFR determination are sample pretreatment, extraction, cleanup, and instrumental analysis (see Fig. 2 for an overview). Laboratory contamination during each analysis step can easily occur due to the presence of BFRs in various indoor consumer products and building materials.

2.1 Extraction

For solid samples, including soil, sediment, sewage sludge, adsorbent materials used for air sampling, and biological tissues, the extraction efficiency depends on the analyte solubility in the extraction solvent, the accessibility of the extraction solvent to the matrix, and the extraction time. The use of binary (nonpolar and polar) solvent mixtures is ideal to obtain high recoveries for BFRs. Extraction techniques, such as ultra-sonication, Soxhlet, liquid–solid extraction, pressurized liquid extraction, or microwave-assisted extraction, are currently applied [18, 21]. Some of these techniques allow lower solvent consumption, which make the long-term costs lower and the procedures more environmentally friendly. For liquid samples, liquid–liquid extraction was applied by using a binary mixture of solvents in river and seawater samples, human milk, or serum. Solid-phase extraction (SPE) has been used mostly for the analysis of neutral and phenolic-type BFRs from

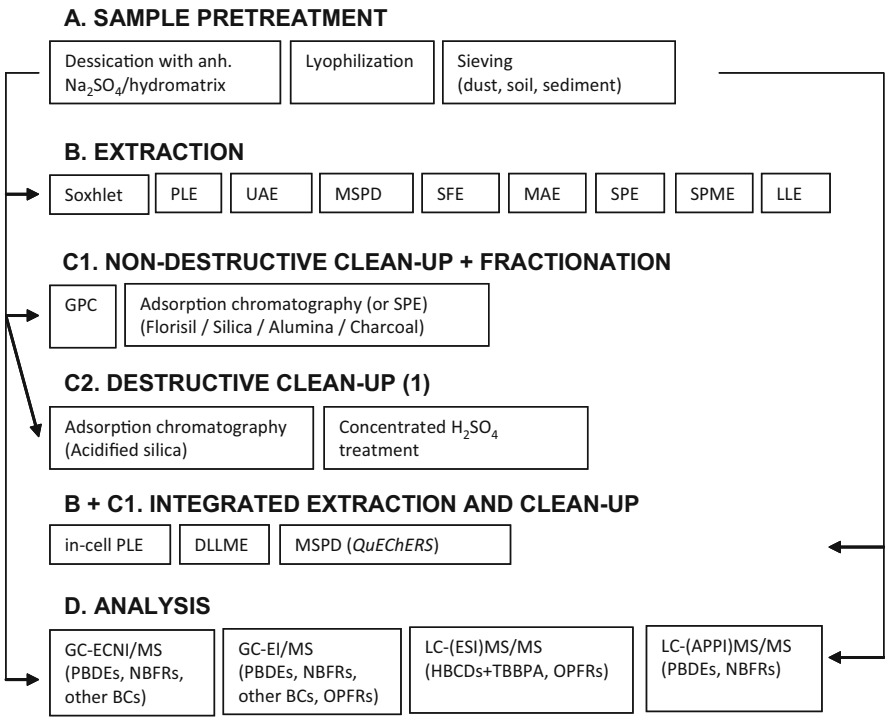

Fig. 2 Simplified overview of the most common analytical flow charts used for the determination of brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs), tetrabromobisphenol-A (TBBP-A), and new BFRs. (1) – destructive cleanup can be applied only for PBDEs, HBCDs, TBBP-A, and some NBFRs. Reproduced with permission from Dirtu et al. [21]

human serum or milk. In most cases, an additional cleanup step is necessary due to high amounts of co-extracted lipids.

2.2 Cleanup

The nonselective nature of the exhaustive extraction procedures and the complexity of the sample matrices result in complex extracts that require further purification prior to analysis. The cleanup techniques for BFRs were recently reviewed [18, 21]. For sediment, sewage sludge, and soil samples, sulfur can be removed from raw extracts by Cu powder or gel permeation chromatography (GPC). For biological samples, co-extracted lipids may be eliminated using either destructive or nondestructive methods. The common nondestructive methods of lipid removal are GPC and adsorption chromatography on selected sorbents. For complex matrices, GPC followed by further adsorption chromatography is often required for complete

fat removal and/or isolation of BFRs from other POPs. Silica, alumina, and Florisil with different degrees of deactivation have been widely used for lipid removal. The most used destructive treatment in BFR analysis is treatment with sulfuric acid by direct addition of the acid to the sample extract dissolved in n-hexane or the use of silica impregnated with sulfuric acid. The use of acidified silica avoids the emulsion problems, reduces the sample handling and solvent consumption, and increases the sample throughput.

2.3 Instrumental Analysis

Because of their physicochemical properties, gas chromatography (GC) has become a standard analytical separation method for PBDEs, while separation based on liquid chromatography (LC) is required for the separation of individual HBCD isomers and TBBP-A.

2.3.1 Gas Chromatography–Mass Spectrometry

The most common injection techniques are split–splitless injection, on-column injection, and programmable temperature vaporization injection. Their advantages and disadvantages derive primarily from their availability, price, acceptable detection methods, and discrimination of congeners on the basis of molecular weight.

Single-capillary column GC may offer sufficient resolution for a congener-specific determination. Sufficiently long columns (30–50 m) with internal diameters below $\leq$0.25 mm are necessary for good separation. Several co-elutions are reported in the literature for BFR analysis, but most of them can be solved by MS detection [18, 22]. Comprehensive two-dimensional gas chromatography (GC $\times$ GC) has been used only rarely for the analysis of PBDEs [23] with μ-ECD or time-of-flight mass spectrometry (TOF-MS). A different approach has to be used for BDE-209, because of its higher instability and degradation at higher temperatures. The GC column should be relatively short (10–15 m) and have low film thickness ($<$0.20 μm) to reduce the elution time [18]. Total HBCD can be analyzed by GC-MS, but no distinction can be made between the contributions of each HBCD isomer [18]. The individual HBCD isomers can be resolved only by LC-MS (see below). The analysis of phenolic-type BFRs, such as TBBP-A, would require derivatization prior to GC analysis. While more laborious than LC-based methods, GC analysis after derivatization results lower limits of quantification. These are more appropriate for measuring TBBP-A at lower concentrations in biological samples [24].

Mass spectrometers (MS) are the most widely used detectors and are classified into low-resolution (LR) or high-resolution (HR) instruments. The LR-MS instruments are operated either in electron ionization (EI) or electron capture negative ionization (ECNI) mode. For PBDE analysis using EI-MS, the major ions formed

are M^+ and the $[M\text{-}2Br]^+$ which can be used for their selective identification and quantification [18]. However, EI-MS has less sensitivity for PBDE congeners with more than six bromine atoms. In ECNI, the low-energy electrons generated by interactions between a high-energy electron beam and a moderating gas react with the analytes to form negative ions. ECNI-MS is usually preferred for the analysis of PBDEs, but it is less selective because of monitoring of the bromide ions $[Br]^-$ for all homologue groups, except for nona- and deca-BDE for which brominated phenoxy ions can be formed. Instead, ECNI-MS is a very sensitive method with one order of magnitude lower limits of detection. ECNI-MS and EI-HRMS are the preferred techniques for the analysis of low-concentration samples, such as human serum and milk.

2.3.2 Liquid Chromatography–Mass Spectrometry

LC-MS and LC-MS/MS offer good results for the analysis of HBCDs and TBBP-A, when using reversed-phase LC coupled to ESI or atmospheric pressure chemical ionization (APCI-MS). However, the use of LC-ESI-MS/MS results in better performance than LC-APCI-MS/MS when the MRM $[M–H]^-$ (m/z 640.6) → $[Br]^-$ (m/z 79) is used for HBCD [25]. Also, the use of methanol and water as mobile phases can be more advantageous for the quantitative analysis of TBBP-A. Using atmospheric pressure photoionization (APPI), PBDEs ionize well in both negative (higher sensitivity for penta- through deca-BDE congeners) and positive (higher sensitivity for di- through penta-BDE congeners) modes and can be thus analyzed also by LC-MS/MS [26, 27].

2.3.3 Quality Assurance/Quality Control

As a general rule, approximately 15% of the analysis time should be spent on the QA procedures. Quantification procedures based on internal/surrogate standard addition are mandatory to compensate for the losses throughout the analytical procedure. While the use of ^{13}C-labeled compounds is recommended, we acknowledge that this is not possible for the analysis of PBDEs by GC-ECNI-MS, except for BDE-209. Alternatively, F-PBDEs can be used as adequate internal standards. The analyte recovery, the use of procedural blanks, and the determination of limits of detection and quantification should be performed for each compound and matrix to be investigated. Standard reference materials (SRMs or CRMs) should be complementarily used for method validation. The external quality control is usually assessed through the participation in interlaboratory tests which facilitates the evaluation and assessment of the overall method performance.

3 Environmental Levels and Trends

An increasing numbers of papers, including several reviews [28–30], have been published in the last decade showing that BFRs can be now measured in various environmental and biological matrices. The concentration of PBDEs, HBCDs, and TBBP-A is expressed in ng/g lipid weight (lw), unless otherwise specified. Total dietary intake for adults is expressed in ng/kg bw/day.

3.1 Fish, Shellfish, and Benthic Invertebrates

Aquatic organisms are good bioindicators of environmental pollution because they concentrate bioaccumulative pollutants in their bodies from water and sediment, in addition to dietary uptake. Various suspected BFR sources in the Western Scheldt estuary, such as production site of BFRs at Terneuzen, Antwerp harbor, or textile industry along the Scheldt, were evidenced by measuring PBDE congeners in biota, including crab, shrimp, starfish, and fish [31]. Concentrations observed in the Scheldt estuary samples were up to 30 times higher than in those from the North Sea, with an increasing gradient toward Antwerp. Verslycke et al. [32] determined PBDEs, HBCD, and TBBP-A in shrimp from the Scheldt estuary (the Netherlands). At the three sites sampled, concentrations in shrimp were PBDEs (2,100–3,560 ng/g lw), HBCD (562–727 ng/g lw), and TBBP-A (0.8 to <7.7 ng/g lw). In 11 species of fish from the River Scheldt in Belgium, Roosens et al. [33] reported that levels of PBDEs and HBCDs were 10 times higher than those usually reported for freshwater systems, indicating local point sources. Eels showed a considerable decrease in levels of both PBDEs and HBCDs from 2000 to 2006, but a strong spatial variation, also confirmed by a subsequent study [34].

Blais et al. [35] determined PBDEs in fish from 11 lakes along an elevation transect in the French Pyrenees. PBDE concentrations were considerably lower than those in fish from other parts of the world. Gomara et al. [36] studied PBDEs in fish and shellfish collected from retail markets in Spain during 2003–2005 and found high detection frequencies of the higher brominated congeners BDE-184, BDE-191, BDE-196, BDE-197, and BDE-209.

Fish from the Great Lakes [37] showed large increases in concentrations of four PBDE congeners from early to mid-1980s with fairly consistent doubling times (generally 2–4 years). In fish samples collected recently, the accumulation rates were slowing and concentrations of penta- and hexa-BDE congeners started to decrease in the mid-1990s. In trout from the Great Lakes sampled during 1980–2009, Crimmins et al. [38] recorded decreases in PBDE concentrations after 2000–2001 in Lakes Huron, Michigan, and Ontario. In Lakes Erie and Superior, concentrations seem to have stabilized, but not yet started to decline significantly.

Shaw et al. [39] determined PBDEs in seven species of teleost fish comprising the major prey items of harbor seals in the NW Atlantic. PBDE and HBCD

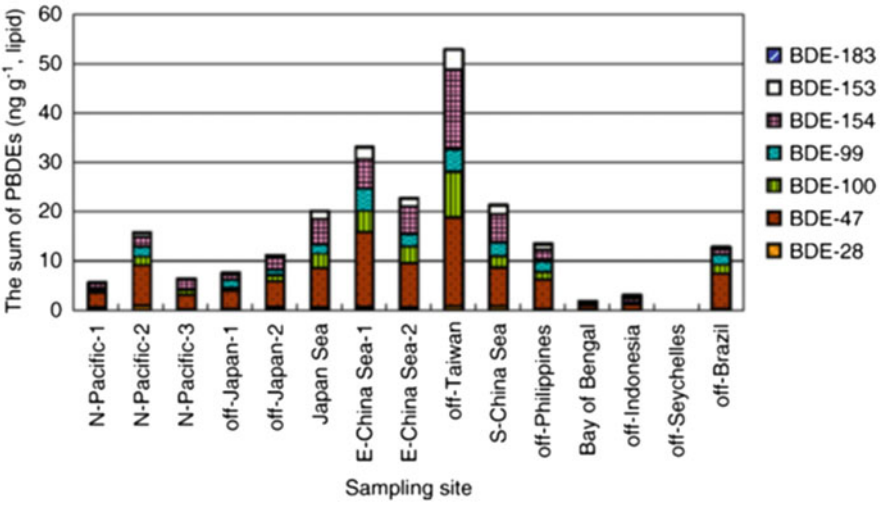

Fig. 3 PBDE concentrations (ng/g lipid weight) in the muscle of skipjack tuna collected from Asian offshore waters, off Seychelles, off Brazil, and in open sea. Reproduced with permission from Ueno et al. [42]

concentrations in whole fish samples (as eaten by seals) ranged from 18 to 94 and 2.4 to 38 ng/g lw, respectively. Losada et al. [40] determined PBDEs in six species of marine fish, crab, and squid from Sydney Harbour, Australia. Mean PBDE concentrations ranged from 6.4 ng/g lw in squid to 115 ng/g lw in flounder. Harrad et al. [41] measured HBCDs (range 14–290 ng/g lw) and TBBP-A (<0.3–1.7 ng/g lw) in fish collected in 2008 from nine English lakes.

Ueno et al. [42, 43] investigated the geographical distribution of PBDEs and HBCDs in the Asia-Pacific region using skipjack tuna (*Katsuwonus pelamis*) as a bioindicator. PBDEs and HBCD concentrations ranged from <0.1 to 53 ng/g lw and <0.1 to 45 ng/g lw, respectively, which are lower than those reported for other studies (Fig. 3). Levels of PBDEs and HBCDs were apparently higher in the northern than the southern hemisphere, probably relating to greater HBCD usage in the northern hemisphere (97% of the total usage in 2001). PBDE levels ranged from 0.8 to 120 ng/g lw in mussels collected in coastal waters of the Asia-Pacific region [44]. The highest concentrations were found in samples from the Pearl River Delta, Hong Kong, which is home to electronics industries, as 30% of the world's computers are assembled in this region.

3.2 Marine Mammals

Law et al. [45] determined 10 PBDE congeners in the blubber of marine mammals of 12 species stranded in the UK between 1992 and 2002. The highest PBDE

concentration (23,800 ng/g lw) was seen in a killer whale, which was feeding at a higher trophic level than the other species [46]. Zegers et al. [47] indicated that all harbor porpoises and common dolphins from Western European seas contained only the α-HBCD isomer. This is in line with its dominance in prey species, e.g., fish [48].

Law et al. [49, 50] reported concentrations of PBDEs and HBCDs in the blubber of harbor porpoises (*Phocoena phocoena*) stranded or died due to physical trauma in the UK during the period 1994–2003. Median sum of PBDEs peaked around 1998 and have since reduced by ~60% to 2008. For HBCDs, a sharp increase has been seen from about 2001 onward, probably as a result of changing usage patterns of FRs following restrictions of penta- and octa-BDE formulations within the EU. However, HBCD levels have dropped after 2004, mirroring the trends in the UK sales of HBCD (Fig. 4, [51]). Levels of TBBP-A in the same porpoises have been shown to be low or undetectable [49].

PBDEs were reported in archived northern fur seals from the Pacific coast of Japan between 1972 and 1998 [52]. The PBDE residual levels were higher than those in the Canadian Arctic [53], but lower than those in San Francisco [54] and Europe [55]. The percentage of higher brominated PBDEs had increased since 1972, whereas the percentage of some lower brominated PBDEs had decreased, which fits with the increase in the use of deca-BDE mixture between 1972 and 1998 [52].

The geographical distribution of PBDEs and HBCDs in small cetaceans from Asian waters was reported based on samples collected from 1990 to 2001 and archived in the Environmental Specimen Bank for Global Monitoring (es-BANK) [56]. The PBDE levels ranged from 6 to 6,000 ng/g lw, while HBCD concentrations were <1 ng/g lw in 1972 and rose to a maximum of 67 ng/g lw in 1997. PBDE

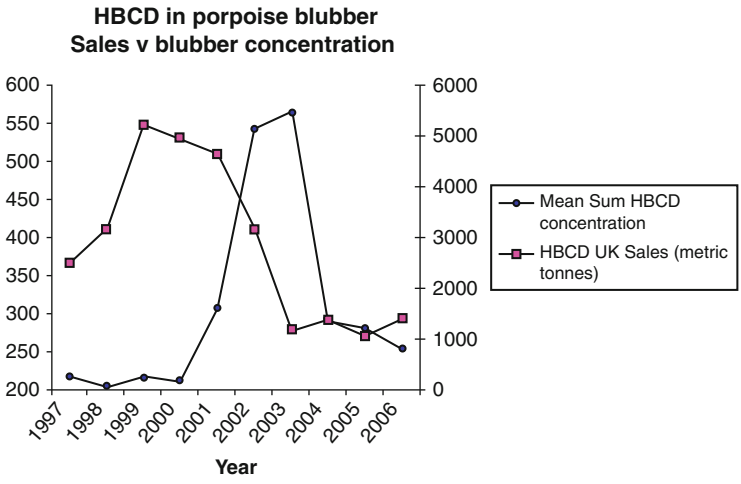

Fig. 4 Plot of mean sum HBCD concentrations (in ng/g) in blubber of harbor porpoises vs. UK sales of HBCD in metric tons, 1997–2006. Reproduced with permission from Law et al. [51]

concentrations appeared to fall after 1994, following a voluntary withdrawal of the penta-BDE formulation in Japan in 1991.

Tanabe et al. [44] investigated the temporal trends of BFRs in Asian waters using samples archived from the es-BANK. In the samples from Japan, where usage of some commercial PBDE products was voluntarily discontinued in the 1990s, PBDE levels seem to be steady or slightly decreasing since then. However, in the same samples, concentrations of HBCDs exhibited a continuously increasing trend, and, in recent years, the HBCD levels appear to exceed those of PBDEs. Increasing environmental contamination by PBDEs and HBCDs was also noticed in Chinese samples, indicating that the contamination by BFRs has already become evident even in developing countries.

Rotander et al. [57] reported time trends of PBDEs in seven species of marine mammals (long-finned pilot whales, minke whales, fin whales, harbor porpoise, Atlantic white-sided dolphins, ringed and hooded seals) from the Arctic and the North Atlantic taken during 1986–2009. The highest levels were found in samples from the late 1990s to 2000.

3.3 Sediment

Concentrations of PBDEs (not including BDE-209) in three sediment cores from the Pearl River estuary, South China, increased gradually from the bottom (mid-1970s) to the middle layer (later 1980s and early 1990s) followed by more variation in the surface sediments [58]. BDE-209 levels remained constant until 1990 and thereafter increased exponentially, with doubling times of approximately 4.5 years, reflecting the increasing market demands for the deca-BDE product in China after 1990. In sewage sludge samples from Korea [59] and Italy [60], BDE-209 dominated the PBDE profile. In Chicago (USA) from 1975 to 2008, penta-BDE concentrations increased initially and leveled-off around 2000 (production of the penta-BDE product ceased in the USA in 2004) [61]. Concentrations of BDE-209 in biosolids rose from 1995 to 2008, doubling every 5 years, another indication of the size of the environmental reservoir of BDE-209 which has been created.

3.4 Terrestrial Environment

Analysis of UK archived pasture (collection period 1930–2004) resulted in the following PBDE trends [62]: (a) PBDEs could not be detected in the pre-1970 samples; (b) a rise through the 1970s; (c) a peak in the mid-1980s, strongly influenced by one particularly high sample for 1984; (d) values remaining high through the late 1980s/1990s; and (e) an indication of a more recent decline for all PBDEs, consistent with restrictions on the usage of the penta- and octa-BDE

mixtures in Europe. Sellstrom et al. [63] determined PBDEs in earthworms collected in 2000 in Sweden. Biota–sediment accumulation factors (BSAFs) declined in the increasing order of the molecular size from tetra-BDE to BDE-209, showing that higher PBDEs, including BDE-209, are bioavailable from soils and accumulate in earthworms, presenting an exposure pathway into the terrestrial food web, as suggested by Law et al. [29].

D'Have et al. [64] investigated relationships between concentrations of PBDEs in hair and internal tissues (liver, kidney, muscle, and adipose tissue) of the European hedgehog (*Erinaceus europaeus*). PBDE levels in hedgehogs probably result from their higher trophic position (insectivorous vs. grazers) in the food chain. Voorspoels et al. [65] measured low PBDE concentrations in tissues of the Belgian red fox (*Vulpes vulpes*). Red foxes are opportunistic feeders, and their diet is diverse, depending on the environment and the time of year. In 40% of the samples, BDE-209 dominated the PBDE profile at concentrations up to 200, 760, and 290 ng/g lw in adipose tissue, liver, and muscle, respectively. This confirms that BDE-209 bioaccumulates in a number of terrestrial top predators, including the red fox.

3.5 Bird's Tissues and Eggs

Fangstrom et al. [66] and Karlsson et al. [67] showed that PBDE contamination in eggs, fat, and muscle tissue of fulmars (*Fulmarus glacialis*) collected in 2000–2001 and 2003 from the Faroe Islands was limited (range 16–43 ng/g lw). Unhatched eggs of peregrine falcons (*Falco peregrinus*) from Madrid and Guadalajara, Spain, collected in 2000–2001 were dominated by BDE-153 and BDE-99 [68], as compared to BDE-47 which is dominant in piscivorous birds. Differences in exposure patterns and in metabolic capacity possibly explain the congener patterns. Dauwe et al. [69] and Van den Steen et al. [70] indicated that PBDE concentrations in Belgian great tit (*Parus major*) eggs were 4 to 6 times higher than those in nestlings, corresponding to concentrations predicted by a bioenergetics-based model. Approximately 60% of the PBDEs in 15-day-old nestlings were still of maternal origin. Van den Steen et al. [71] also studied BDE-209 biotransformation in starlings (*Sturnus vulgaris*) following exposure using implants. No lower brominated congeners could be detected in the blood; however, octa- and nona-BDEs were detected in the liver and, to a lesser degree, muscle.

Jaspers et al. [72] and Kunisue et al. [73] showed the widespread in the PBDE patterns between aquatic and terrestrial predatory birds (Fig. 5). Watanabe et al. [74] determined PBDEs in the livers and eggs of common cormorants from Japan sampled in 2000. BDE-47 comprised 40–50% of the total PBDE content in both liver and eggs. PBDE concentrations were lower than those in cormorants from the UK and the Netherlands. Chen et al. [75] determined BDEs in birds of prey from northern China. BDE-209 was the dominant congener in tissues from some buzzards, scops owls, and little owls. Most of the raptors studied were from

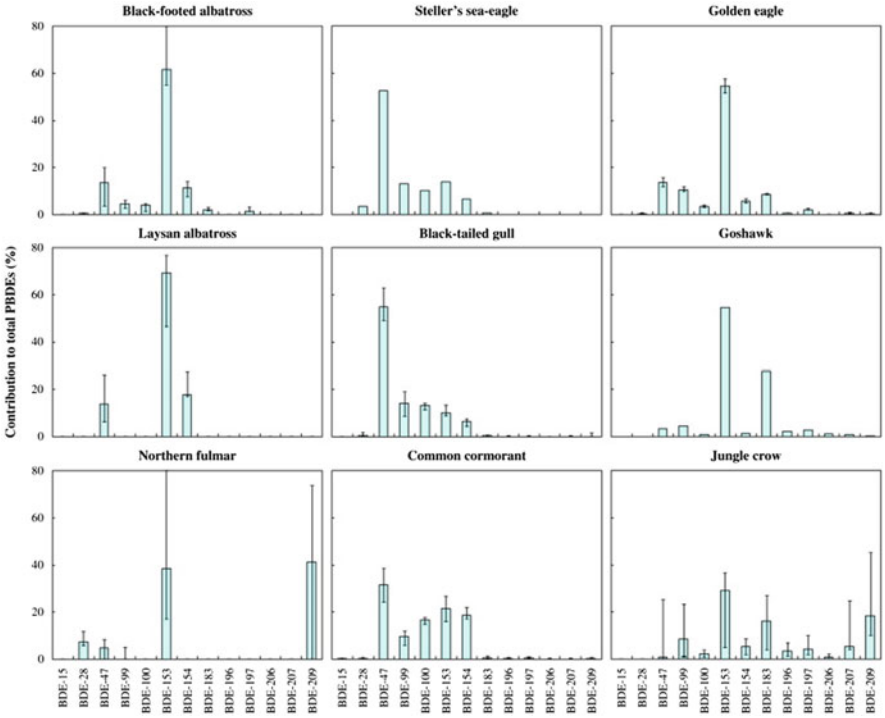

Fig. 5 Composition of PBDEs in avian species from Japan. Reproduced with permission from Kunisue et al. [73]

relatively urbanized areas and may have been exposed via contact to flame-retarded products and/or via their diet. These results support the hypothesis that terrestrial food chains have greater exposure to BDE-209.

In contrast to aquatic species, no time trends could be established for HBCDs in terrestrial birds, such as peregrine falcon and sparrow hawk from the UK sampled between 1973 and 2002 [76] or in peregrine falcon from South Greenland sampled between 1986 and 2003 [77]. Interestingly, for the latter species, a 10% increase per year in the PBDE levels has been observed throughout the investigated time period.

Several studies have reported on the isomeric HBCD pattern in bird tissues, with the dominance of α-HBCD apparent in many, but not all birds [78]. The highest concentrations of HBCDs have now been observed in a peregrine falcon egg from Montreal, Canada, collected in 2007 (sum HBCDs 14 600 ng/g lw; [78]), with α-HBCD dominating, and in sparrow hawk muscle collected in 1995 from the UK (sum HBCDs 19,000 ng/g lw; [79]), with α-HBCD dominating. Important review papers describing BFR concentrations and trends in birds have been published recently: global trends [80] and BFRs in birds and other wildlife from China [81] and the Arctic [82, 83] and in US birds [84].

Several studies have concluded that the geographical patterns and trends of PBDEs and HBCDs measured in the eggs of peregrine falcons [78, 82], ospreys [85], herring gulls [86], and passerines [87] reflect continental usage patterns and human population densities and/or introduced legislation governing their manufacturing and usage. Other studies have concluded that sources of PBDE exposure as reflected in bird eggs have changed over time [88]. Median PBDE concentrations of bird tissues were similar regardless of diet, ecosystem, or continent, contrasting with continental differences in aquatic birds reported over a longer time period by Chen and Hale [80].

3.6 Arctic and Antarctic

In adipose tissue of polar bears from western Hudson Bay, sampled at intervals between 1991 and 2007, PBDE concentrations showed significant increases of 13% per year between 1991 and 2007 [89].There were differences in the relative proportions of BDE-47 and BDE-153, with adult males having lower proportions of BDE-47 and higher proportions of BDE-153 than adult females and subadult bears, which reflect dietary differences and the role of lactation on dietary release by females and uptake by subadults [89]. During the 1990s, α-HBCD was not detected, but, after 2000, it was detected at concentrations 5–10 times lower than those of PBDEs, with a maximum concentration observed in 2003.

Muir and de Wit [90] reported evidence that environmental levels of penta-BDE-related congeners were leveling off or beginning to decline in Arctic samples. They reviewed studies of various fishes (Arctic char, burbot, trout), birds (northern fulmar, thick-billed murres, guillemots), and marine mammals (ringed seals and beluga) and concluded that most studies indicated declining concentrations of BDE-47 and BDE-99. Most surprising was the predominance of BDE-209 in air samples, all of it in the particle phase.

4 Food Chain Studies

BFRs are chemically and biologically persistent and furthermore lipophilic, which results in their bioaccumulation in fatty tissues of organisms and biomagnification throughout food chains [28, 29].

Bragigand et al. [91] indicated that the major biomagnification step for PBDE congeners in the food webs of two major French estuaries was between primary consumers and omnivores. Magnusson et al. [92] observed no biomagnification of ^{14}C-BDE-99 in the marine copepod *Calanus finmarchicus* exposed to contaminated water or after being fed on contaminated plankton. The importance of individual interactions and the kinetics of bioaccumulation at the bottom of the food chain were highlighted. Burreau et al. [93] showed that biomagnification of PBDEs

occurred similarly in food chains from the Baltic Sea and the North Atlantic Ocean, meaning that the ratio between a prey and its predator is the same in spite of different concentrations.

van Beusekom et al. [94] modeled the accumulation of PBDEs and HBCDs in a benthic fish (barbel) and a pelagic fish (bleak) from the Ebro River, Spain. The model accounted for BFR uptake from water, food, and ingested sediment; release via water and feces; growth; and in situ binding of BFRs to black carbon. The higher BSAFs in barbel compared to bleak were explained by differences in age, growth, feeding behavior, and uptake through sediment ingestion. Concentrations of PBDEs in aquatic species from the Scheldt estuary were related with factors (body size, lipids, trophic position) possibly influencing their bioaccumulation [95, 96]. While a decreasing trend in PBDE levels toward the North Sea was observed, biomagnification was more important in the marine part of the estuary.

Baek et al. [97] indicated that α-HBCD was the dominant isomer in two marine food webs in Norway (Oslofjord and Svalbard) and that it showed a high potential for biomagnification. In the Oslofjord, levels increased from invertebrates to fish, but not from fish to harbor seals. In Svalbard, HBCD biomagnified in the food chain to ringed seal, but not from ringed seal to polar bear, indicating that polar bears can metabolize α-HBCD. Muir et al. [98] demonstrated that BDE-47, BDE-99, BDE-100, and BDE-153 substantially biomagnified from seals to polar bears with biomagnification factors (BMFs) ranging from 3.9 to 71. Lundstedt-Enkel et al. [99] predicted the biomagnification of PBDEs and HBCDs in Baltic Sea biota using data from herring muscle and guillemot eggs. A good agreement was seen between the observed and predicted BMFs, and the model could be used to predict BMFs for novel FRs.

The biomagnification potential of PBDEs was assessed in terrestrial food chains by using data on passerines, rodents, and terrestrial predators, e.g., birds of prey and foxes [65, 72, 100, 101]. BMF values for PBDEs in buzzard and sparrow hawk ranged from 2 to 34, depending on the congener, indicating biomagnification. Surprisingly, no biomagnification was observed from rodents to foxes, and the median sum PBDEs in fox was even lower than in rodents, most probably due to extensive metabolism in foxes. Muscle of kingfishers (*Alcedo atthis*) and their prey fish species from an electronic recycling site in south China were assessed for the concentrations and biomagnification potential of PBDEs (17 congeners) [102]. Levels of sum PBDEs in kingfishers (range 2,030–26,400 ng/g lw) were higher than those in the muscles of seven water bird species (range 37–2,300 ng/g lw) and three terrestrial species (1,000–5,200 ng/g lw) from the same site [103, 104]. BMFs were >1 for all PBDE congeners, except BDE-28, with mean BMFs of 1.4 to 4.5 depending on the fish species [102].

The characterization of food webs makes the behavior of BFRs in the environment more understandable, since trophic magnification of persistent compounds can lead to increased exposure and consequently to toxic effects in organisms at the top of the food chain. The potential risks to human exposure are of special concern considering our omnivorous diet.

5 Human Exposure

Based on recent studies, it seems that human exposure of the general population to PBDEs (and BFRs in general) occurs mainly via a combination of diet, ingestion of indoor dust, and inhalation of indoor air [105].

5.1 Dietary Intake

Due to their lipophilic nature, BFRs are mostly found in lipid-rich food of animal origin, such as meat, fish, eggs, and dairy products. The human dietary intake of the sum of PBDEs ranges from 28 to 42 ng/day for Ireland [106], 49 ng/day for Sweden [107], 51 ng/day for the Netherlands [108], to 98 ng/day for Norway [109]. Domingo et al. [110] estimated in Spain a dietary intake of sum PBDEs of 75 ng/day from 11 food groups, an intake lower than that previously reported (97 ng/day) for the same region by Bocio et al. [111]. In Belgium, the dietary intake of PBDEs was also low, 23–48 ng/day (lower–upper bound) [112], and comparable to other European countries. Clearly, dietary exposure to PBDEs in Europe ranges between 0.5 and 1.5 ng/kg bw/day as calculated for an adult of 70 kg.

A common conclusion of most dietary exposure studies is the high contribution of fish, seafood, and dairy products to the total dietary exposure to PBDEs and the probably limited human health risks derived from dietary exposure, accepting the suggested US EPA reference dose values (http://www.epa.gov/iris). In the USA, meat products are a major contributor to PBDE intake [113]. EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) [114] delivered a scientific opinion on PBDEs in food based on data from 11 European countries and concluded that for BDE-47, BDE-153, and BDE-209, the current dietary exposure in the EU does not raise a health concern. However, it was stated that there is a potential health concern for BDE-99 with respect to the current dietary exposure needed.

The EFSA CONTAM Panel reviewed the dietary exposure to HBCDs across dietary surveys in European countries [115]. The intake for 3–10-year-old children ranged from 0.15 to 1.85 ng/kg bw/day. Total dietary exposure for adults (0.09–0.99 ng/kg bw/day) was about 50% of the children's exposure. Dietary exposure to HBCDs decreased with increasing age down to 0.06–0.54 ng/kg bw/day, for adults above 75 years. In Belgium, the average dietary exposure to the sum HBCDs was 0.99 ng/kg bw/day, range 0.8–1.18 ng/kg bw/day [116]. The meat group was the highest contributor (43%) to the estimated average daily intake. Beef was the main contributor within that group with 48% followed by prepared meat with 22%. Surprisingly, the most contaminated group, fish and fishery products, accounted for only 7% of the daily intake. Within this group, fatty fishes (salmon, herring, and sardines) were the main contributors. HBCD levels in US food were low, consistent with its usage trends, with the highest levels in canned fish [113].

A dietary exposure assessment for TBBP-A was performed by the EFSA CONTAM Panel on 344 food samples from Norway and Spain [117]. A worst case intake estimate for the specific group of adult high fish consumers resulted in an "upper bound" intake estimate of 2.6 ng/kg bw/day. The Panel concluded that current dietary exposure to TBBP-A in the EU does not raise a health concern, neither does the exposure of infants via human milk.

Ashizuka et al. [118] detected TBBP-A in 29 out of 45 Japanese fish samples at <0.11 ng/g ww. The mean level of TBBP-A was about $<1/10$ of the total level of PBDEs. The daily intakes from fish were estimated to be 0.03 ng/kg bw/day for TBBP-A. Murata et al. [119] analyzed two sets of market basket food samples from Japan in 2002 and 2005. The dietary intakes of HBCD, TBBP-A, and PBDEs for an adult were 2.2, 1.1, and 2.3 ng/kg bw/day, respectively, in 2002 and 1.4, 0.1, and 1.4 ng/kg bw/day, respectively, in 2005, indicating overall decreasing concentrations.

It is important to mention that a number of issues have only very recently been investigated, such as bioaccessibility of ingested food [120], changes in concentrations and patterns of BFRs during cooking processes [121], and modeling of BFR dietary exposure [106, 122]. It is expected that they will continue to grow in importance in the next few years.

5.2 Indoor Dust

Due to low vapor pressures, BFRs will preferentially partition indoors to dust, and, therefore, human exposure to BFRs is better assessed through ingestion of settled dust, rather than via inhalation of indoor air.

Levels of PBDEs and HBCDs in house dust from several countries (Canada, New Zealand, UK, and USA) were assessed by Harrad et al. [123] and Abdallah et al. [124]. The results show that North American dusts are contaminated by both deca- and penta-BDE commercial formulations; penta-BDE in US dusts have approximately 10 times higher concentrations than UK dusts, which in turn are contaminated predominantly by deca-BDE and HBCDs. The octa-BDE formulation appeared of minimal importance in accordance with available market demand figures. Although commercial PBDE formulations have never been manufactured in nor imported into New Zealand, the presence of PBDEs in NZ dust suggests that international trade in PBDE-containing goods is an important pathway effecting their global distribution. These trends have been afterward confirmed by a multitude of research studies reviewed by Harrad et al. [105] and Besis and Samara [125].

In parallel, levels of BFRs have been reported in dust from countries for which such information has been hitherto unavailable. These studies include dust from homes and cars in the Czech Republic [126], homes and computer offices in Poland [127], and homes in Romania [128]. Concentrations of PBDEs and HBCDs in Central and Eastern European indoor dust were similar or at the low range of

Western European levels, thereby providing some evidence that dust ingestion may be an important contributor to the human exposure also in these countries. TBBP-A had much lower concentrations in dust, leading to a lower human exposure to TBBP-A [105, 129].

While regional and national variability in BFR levels in dust exists and concentrations in developing and transition economies are often lower than those in developed regions, the burgeoning database serves to emphasize the ubiquitous indoor presence of BFRs. It also appears that diet becomes an important exposure route only when dust levels of BFRs and thus the contribution of dust to total exposure are low [130]. The importance of each exposure route might be country specific.

5.3 Biological Matrices

Recent studies have described PBDEs and/or HBCD temporal trends (1993–2009) in human matrices, such as human serum and milk. For PBDEs, decreasing temporal trends and a peak followed by plateau were identified in Australia [131], Italy [132], Germany [133, 134], and the USA [135]. In contrast, PBDE concentrations increased over time in Guinea-Bissau (West Africa) [136] and Ghana [137]. HBCD concentrations, where reported, were more variable, without any temporal trend [131, 137]. Points of interest in these studies were an increase in BDE-209, a congener rarely reported in humans, in children from Baden-Württemberg, Germany, from 2002 to 2009 [134], and an increase in the proportion of BDE-153 compared to the previously dominant BDE-47 [133, 135, 136].

An earlier meta-analysis of PBDE levels [138] has shown that PBDE levels in humans have increased over the 30 years preceding the review. Biological samples from North America were always above the regression line (in recent years by a factor of >10) and that the Japanese samples are usually below the regression line (by a factor of ~5) showing geographical differences in the PBDE exposure levels. Different PBDE profiles were observed comparing occupationally exposed (with higher levels of PBDEs with 7–10 bromine atoms) and nonoccupationally exposed populations (with higher levels of PBDEs with 3–6 bromine atoms) (reviewed by [139]).

Concentrations of PBDEs in serum from US children have been positively associated with the PBDE levels in maternal serum during pregnancy, duration of breast feeding, and socioeconomic status [140, 141]. Levels of penta-BDE congeners in Danish dust were significantly correlated with those in both matched air and placenta samples, indicating indoor exposures may be an important pathway of exposure for these PBDEs, but not for BDE-209 [142]. Combined, these studies add weight to the hypothesis that indoor contamination can be an important driver of human body burdens of BFRs.

5.4 Modeling Approaches

When information is available regarding multiple exposure pathways to BFRs for a specific population, modeling approaches can be useful to rank the relative important of these exposure pathways. The first approach to model the human exposure to PBDEs was developed by Lorber [143]. After compilation of the available exposure media data on PBDEs, an adult intake dose was derived using exposure factors in combination with these data. The exposure pathways evaluated included food and water ingestion, inhalation, and ingestion and dermal contact to house dust. These intakes were converted to a body burden using a single compartment pharmacokinetic (PK) model, and the predicted body burdens were compared with representative profiles of PBDEs in blood and milk. An important finding was that body burdens of the US population could not be explained by the food intake, while exposures to PBDEs in house dust accounted for 82% of the overall estimated intakes [143].

Using a similar approach, human exposure to PBDEs and HBCDs has been assessed in Belgium by a combination of BFR measurements in relevant exposure media (food, dust, and human milk) and compilation of literature data for the missing information [130]. The exposure model covered human exposure of infants, children, and adults through human milk, food, dust/soil ingestion, and air inhalation. The estimated human exposure was at the low end of what has been reported in previous European intake assessments and was lower than the estimated US exposure.

Other exposure models have been developed to assess the exposure of the Irish population to PBDEs in food [106, 122] and have concluded that there is no significant risk for human health through intake of PBDE from food. Interestingly, the authors have indicated that the datasets used for the model contained little uncertainty and that additional measurements would have not significantly improved the quality of dose estimates. The Irish intakes of PBDEs appear to be comparable to intakes derived for Belgian and Dutch adults [122].

Using a simple one-compartment pharmacokinetic model, PBDE intakes of UK adults via inhalation, diet, and dust ingestion were converted to predicted body burdens [144]. Predictions compared well with those observed for Σtri-hexa-BDEs and BDE-209 in breast milk.

Modeling approaches have also been used to examine the balance between intake, intrinsic elimination half-lives, and human body burdens measured in biomonitoring for PBDEs in the North American population [145]. The results have indicated inconsistencies between the PBDE intake estimates and the biomonitoring data, which is likely due to underestimation of the PBDE intake at population level. Additional age-stratified biomonitoring data and time trends of PBDE intakes would improve the model and provide a better estimation of the intrinsic elimination half-lives.

6 Toxicology of BFRs

While information regarding levels of BFRs in the environment and humans is abundant, we are still lacking relevant information on their health effects [4, 146]. Generally, PBDEs, HBCDs, and TBBP-A are absorbed from the gastrointestinal tract [120] and accumulate in fatty tissues. While not causing immediate symptoms from acute toxicity at average doses, the health effects from chronic exposure to BFRs are of more concern, especially when they are related to the exposure of developing infants and wildlife.

Even if limited information is published in this field, there is some evidence that BFRs can cause developmental effects, endocrine disruption, immunotoxicity, and reproductive, behavioral, and long-term effects, including second-generation effects [4, 146–148]. For PBDEs and TBBP-A, there is some evidence available for estrogenic activity [149, 150], but more studies have to be undertaken to determine if low-dose exposures have estrogenic activity in humans or other species. The penta-BDE congeners have been shown to cause toxicity at lower doses than the octa- through deca-BDE congeners [147]. For HBCDs, extrapolation of test results from mammals indicates possible effects on the thyroid, liver, and nervous system [151–153]. Yet, HBCD lacks significant genotoxic potential in vitro as well as in vivo [153]. Recent toxicological advances include a better mechanistic understanding of how PBDEs and HBCDs can interfere with the hypothalamic–pituitary–thyroid axis and impact hormone receptor pathways (e.g., thyroid, estrogen, androgen, progesterone, and AhR receptor pathways [153, 154]). Toxicological studies with TBBP-A have been carried out using different experimental designs with single or repeated administration during gestation, postnatally, or in adulthood. The main target is thyroid hormone homeostasis, while TBBP-A is not genotoxic, and there are no indications that TBBP-A might be carcinogenic [4].

7 e-Waste

Electronic waste (or e-waste) is frequently recycled with primitive technologies such as open burning, resulting in severe human and environmental contamination by BFRs and their combustion products. e-Waste recycling has been intensively performed in Asia resulting in extensive exposure to BFRs at these sites. Consequently, the potential effects of BFRs on the health of local populations have become of increasing concern [155, 156]. A notable number of studies report relatively high PBDE and/or HBCD concentrations in biota from e-waste recycling areas in China [87, 103, 104, 157]. Home-produced chicken and duck eggs produced in the vicinity of e-waste recycling sites in China contained high levels of PBDEs and HBCD [158].

Recent Chinese government actions to limit the importation of e-waste have resulted in attention switching to other Asian countries, such as Thailand and

Vietnam or Africa [157, 159, 160]. Decreasing concentrations of PBDEs have been reported in some studies [156, 161], underlying the beneficial impacts of these Chinese government actions. Future research should focus on a better characterization of the impacts of BFRs on human exposure and health and on improving the knowledge regarding such informal e-waste treatment activities in hitherto understudied regions, e.g., Africa. Also, the formation and release of brominated and mixed brominated/chlorinated dioxins and furans during these activities and their possible health effects needs to be better documented [162–165].

8 Data Gaps and Trends

A recent review on BFRs [166] has summarized a number of data gaps which have to be filled in the future and issues which need to be more thoroughly investigated. While restrictions on the use of the penta- and octa-BDE technical mixtures start to yield declines in their environmental levels, major concerns still remain regarding the potential debromination of BDE-209. Given the large amount of deca-BDE in sediments, it is necessary to establish whether terrestrial and marine plants (e.g., plankton, seaweeds) can degrade BDE-209. Furthermore, there is a gap in knowledge concerning the metabolism/debromination of BDE-209 and HBCD and the potential effects of these biotransformation products in biota.

Much knowledge has been gained regarding global concentrations and trends of BFRs in wildlife. Also, there is minimal information regarding the exposure and possible effects of BFRs in amphibians and reptiles.

The time trends of BFRs in human samples need to be followed at country level. Children's exposure to BFRs would be better assessed by direct measurements of serum concentrations, rather than indirectly through estimation of dietary intake and dust ingestion [167]. Since ethical issues for blood have yet to be overcome, there is potential for non- or minimally invasive measures of body burden, such as hair and nails, to be investigated.

BFR contamination at e-waste recycling and dumping sites from Asia and Africa remains of high concern. Continuous monitoring of human exposure and environmental contamination in these regions is warranted. In particular, the formation and release of brominated and mixed brominated/chlorinated dioxins and furans during these activities and their possible health effects needs to be addressed.

Due to the ongoing restrictions on PBDEs and the impending inclusion of HBCDs in the POPs Stockholm Convention, there has been a shift in the use of FRs, with the recent increasing consumption of NBFRs and phosphorus flame retardants (PFRs). Investigation of occurrence and time trends in various environmental media, biota, and human samples for these alternative FRs is strongly recommended.

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Analysis of Chlorinated and Phosphorus Flame Retardants

Chris H. Marvin, Edward Sverko, and Gregg T. Tomy

This chapter is dedicated to Otto Hutzinger, a pioneer in the analysis of dioxins and furans and mentor and inspiration to an entire generation of environmental analytical chemists.

Abstract The bulk of the environmental science on flame retardants (FRs) has been on the brominated compounds (BFRs). However, there are a number of environmentally relevant classes of FRs falling outside the scope of BFRs. These FRs include chlorinated paraffins (CPs), the dechloranes and related series of compounds and phosphorus FRs (PFRs). The non-brominated flame retardants falling within the classifications of PFRs, CPs and the dechlorane series of compounds can require complex sample preparation schemes and analytical procedures using highly sensitive and selective state-of-the-art instrumentation to achieve stringent data quality objectives. Analytical procedures including extraction, cleanup and instrumental analysis are reviewed. Emphasis has been placed on developments since the most recent comprehensive reviews of analytical techniques for these three compound classes.

Keywords Analysis, Blanks, Chlorinated flame retardants, Chlorinated paraffins, Cleanup, CPs, Dechlorane, Extraction, Gas chromatography, Liquid chromatography, Mass spectrometry, PCAs, PFRs, Phosphorus flame retardants, QA/QC, Quality assurance/quality control, Review, Sample preparation, Tandem mass spectrometry

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Contents

1 Introduction

Brominated flame retardants (BFRs) comprise roughly 40% of the global market for FRs and as a result have been subjected to regulatory scrutiny and their occurrence, distribution and fate widely studied in the environment [1]. However, other classes of FRs based on chlorine (~5% of global market) and phosphorus (~40% of global market) collectively comprise roughly half the global FR market that amounts to over 2 billion USD annually. For some of the non-BFRs, there is a paucity of environmental data that is a prerequisite for determining their persistence, bioaccumulative potential, toxicity and amenability to long-range transport (LRT) and therefore their overall environmental relevance. In some cases, there is application for these chemical classes outside their role as FRs; for example, the CPs are also used as high-pressure lubricants and cutting oils, while the PFRs have also found application as plasticizers. Some of the PFRs have also been identified as potential alternatives to BFRs.

This chapter focuses on the determination of three classes of non-BFRs, phosphorus flame retardants (PFRs), chlorinated paraffins (CPs) and the dechlorane class of FRs and related compounds. The properties, production, environmental occurrence, toxicity and analysis of PFRs were the subject of a very comprehensive review by van der Veen and de Boer [2]. Although PFRs have been in use for over 150 years, information on their occurrence, distribution and fate lags behind that for many BFRs. Since publication of this excellent review, there have been significant advances in methods development for PFRs and their associated metabolites in biological matrices, as well as development of comprehensive methods for determination of multiple compound classes, including PFRs. In terms of the CP class of compounds, the chapter focuses on the short-chain (C_{10}–C_{13}) compounds. Analytical methods for CPs and their associated data quality objectives are a critical consideration for this class of compounds as the current lack of regulatory action on these chemicals is due in part to a perceived lack of environmental exposure data of sufficient quality to inform global initiatives such as the Stockholm Convention [3]. This data gap is not surprising given the incredible complexity of the CP

technical mixtures and the corresponding daunting analytical challenge their analyses present. Dechlorane Plus (DP) is a highly chlorinated FR that has been in production for over 50 years, but only considered as a chemical of interest in the past decade. In addition, a number of structurally related compounds arising from Diels–Alder reactions of impurities in DP feedstocks have been detected in environmental compartments [4]. As with the PFRs, DP is a high-production volume (HPV) chemical and could be considered as a replacement for some BFRs, particularly decabromodiphenylether. As a result, robust analytical methods are required for DP and its related compounds in order to accurately measure their environmental fate and behaviour.

The three classes of chemicals covered in this chapter represent a broad range of physical and chemical properties and in some cases are amenable to a number of analytical methodologies. In some cases, such as is the case for the PFRs, analytical methods continue to evolve as metabolites in biological matrices are of increasing interest. For the purposes of this chapter, the analytical methods reviewed are based on separations using high-performance liquid chromatography (HPLC) or high-resolution gas chromatography (GC) using mass spectrometric detection. Mass spectrometric techniques include the use of quadrupole (single or tandem), magnetic sector, time-of-flight (TOF), inductively coupled plasma (ICP) and ion trap instruments in conjunction with an array of ionization techniques. This chapter has been tailored to analysts, and as a result we have separated the descriptions of the analytical methods into discrete subchapters according to compound class.

2 Dechlorane Plus and Dechloranes

Of the organic-based flame retardants (FRs), uses and production of the chlorinated class are by far outweighed by its brominated relatives; the greater labile nature of the carbon–bromine bond lends itself as a more effective FR. However, the chlorine-based commercial products have received recent attention from the environmental community. While in production for over 40 years, Dechlorane Plus (DP) was only first reported on in 2006 [5]. Since then it has been shown to be a worldwide contaminant [6, 7]. The European Commission has identified DP as a possible replacement for the high-production volume flame retardant, decabromodiphenylether, providing the prospect of increased DP usage in the future.

More recently, a group of "DP-like" FRs were detected in the environment in 2010, namely, Dechlorane 602, 603 and 604 (Dec-602, Dec-603 and Dec-604) [4, 8]. Their production and uses are less well known, yet environmental occurrences are shown to be ubiquitous [4, 9–12].

2.1 Analytical Techniques for Dechlorane Plus and Dechloranes

General analytical techniques for these chlorinated FRs have largely been described previously by Sverko et al. [7] and Xian et al. [13]. Many of these extraction methods are synonymous to those used for lipophilic semi-volatile compounds such as organochlorine pesticides and polybrominated diphenyl ethers. The differences reside on the instrumentation, both in detector platform and detection mode. Since the time of the previous reviews in 2011, there have been several publications in the peer-reviewed literature providing alternative approaches to the instrumental portion of the analysis as well as a few novel extraction techniques. Additionally, new dechlorane-related compounds have been reported in the environment. This section will present these new analytical approaches along with those for the newly identified dechlorane-related compounds.

As previously mentioned, the analytical techniques to measure DP and dechloranes are similar to the ones used for persistent organic pollutants. However, it must be stressed that DP was shown to dechlorinate in situ in the liner of a gas chromatograph (GC) in certain conditions [7]. These artefacts normally occur when a gradual, depositional buildup of nonvolatile sample matrix is amassed likely providing an electron-donating substrate for dechlorination. Therefore, it is highly recommended that the *m*/*z* related to DP's [−Cl+H] and [−2Cl+2H] analogues be monitored during the course of the mass spectrometric (MS) analysis. In electron capture negative ionization (ECNI) mode, these *m*/*z* losses would stem from DP's M^- cluster, whereas in the electron impact (EI) mode, the losses would be removed from the abundant $C_5Cl_6^+$ fragment at *m*/*z* 270. Failure to monitor for these in situ [−Cl+H]-type losses will affect the ability to accurately determine the DP isomer fractional abundance (f_{syn}).

Various passive air sampling techniques have been developed over the last decade. These are largely based on uptake rates calculated from depuration studies and various modelling approaches. This technique has become widely used based on its simplistic design and application. Okonski et al. looked at the particle size distribution of DP, among other FRs, and its implication for atmospheric deposition and transport [14]. High molecular weight compounds, such as DP (which also possesses a high log K_{OA} of 14), mean that the isomers will almost entirely be particle bound. When the authors calculated wet and dry deposition using particle size-segregated data, lower deposition estimates were observed than when compared to bulk aerosol data. This suggests that DP has a greater long-range atmospheric transport than previously thought.

Chen et al. applied a new analytical approach for analysing DP and dechloranes in marketed fish using matrix solid-phase dispersion (MSPD) [15]. This technique involved adding an adsorbent, so-called co-sorbent, to a 1 g freeze-dried homogenized fish sample. The mixture of dried sample and co-sorbent was added into a solid-phase extraction cartridge which contained a packed second adsorbent, used as an in situ simultaneous sample cleanup. Once capped with a frit, the target

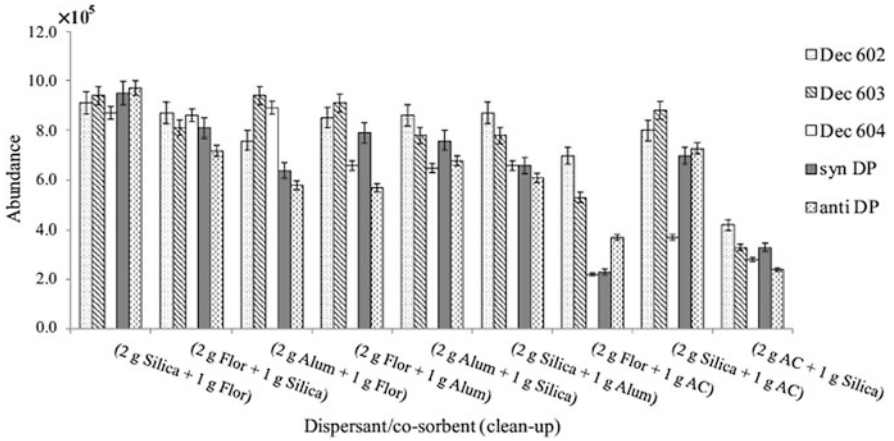

Fig. 1 Recovery efficiencies for various combinations of co-sorbents and cleanup adsorbents [15] (with permission)

compounds were collected using 20 mL of hexane. The authors compared several permutations and combinations of various co-sorbents and cleanup adsorbents (Fig. 1) to determine the best extraction efficiency.

Ultimately, the best co-sorbent/cleanup adsorbent combination was 2.0 g of silica/1.0 g Florisil. This was then compared against the traditional Soxhlet extraction method which showed to be as comparable if not better, giving limits of detection (LOD) of 3–5 pg/g lipid weight. The predominant benefits to this method over traditional methods are the ease, efficiency and low solvent use.

The researchers conducted a screening level study of various fish species available in the market using the newly developed method. They found that both *syn*- and *anti*-DP, Dec-602 and Dec-603, were all detected in their samples ranging from 14 to 373 pg/g lipid weight, while Dec-604 was detected in all but one species, salmon, at a concentration range of 26–41 pg/g lipid weight.

Shi et al. developed a method to analyse DP in various fish oil supplements [16]. The analytical approach was largely conventional using Soxhlet extraction, gel permeation chromatography and analysis by GC/ECNI/MS. During their fish oil surveillance, no DP isomers were detected with detection limits in the low pg/g range.

Zhang et al. reported on a multi-residue analysis of legacy and emerging persistent organic pollutants, including DP, by GC tandem mass spectrometry (GC/MS/MS) [17]. They analysed dissolved seawater and suspended sediment around the Singaporean coastline. Their tandem mass spectrometric approach afforded them a simultaneous analysis of 86 compounds while providing improved specificity. Using the EI mode, the precursor ion was that of the $C_5Cl_6^+$ fragment, *m*/*z* 271.8 with a product ion of 236.9 corresponding to a chlorine loss. The confirmatory precursor/product transition *m*/*z* ions were based on the Cl isotopic ions 273.8/238.9, respectively. The authors' approach to calculating method

detection limits (MDLs) was to derive this number by a 5 times signal-to-noise ratio of the target compound in a sample using a root mean square to calculate the noise. Using this approach, they noted that the *syn*- and *anti*-DP MDL values in both dissolved seawater and suspended sediment were both 0.5 and 0.4 pg/L, respectively; the suspended sediment MDL was total suspended solids adjusted. The total DP concentration range during this research was 7.5–23.9 pg/L. Their subsequent work was a method developed for sediment and biota collected in the same sampling area. Method detection limits for *syn*- and *anti*-DP in sediment were both 0.2 pg/g wet weight, while *syn*- and *anti*-isomer MDLs for biota were 1.2 and 1.1 pg/g dry weight, respectively [18].

Another group presented research using the same tandem MS platform; however, chromatographic separation was conducted by liquid chromatography (LC). Zhou et al. showed that separation of DP and dechloranes in environmental samples could be analysed in 5 min run times [19]. After investigating three atmospheric ionization modes, electrospray ionization, atmospheric pressure chemical ionization and atmospheric pressure photoionization (APPI), APPI was deemed the most sensitive. Various LC columns and mobile phases were investigated to provide the best separation efficiency. Optimal conditions for separation were determined as utilizing a biphenyl column (100 mm × 2.1 mm, 3.0 μm) and a mobile phase comprising of both (A) methanol/water (10:90) and (B) methanol at an A/B ratio of 1:10, with a linear gradient from 10% A to 100% B over a 3-min period. The final 2 min were isocratic at 100% B; acetone was used as the dopant at 0.25 mL/min.

Because the ionization modes compared to the GC-based EI mode were "softer," much like ECNI, the *m*/*z* ions were larger (in the 500–655 range) providing greater specificity than those of lower mass monitored by GC/MS/MS. Limits of quantification on surface water ranged from 2 to 46 ng/L, while for fish and sediment, the authors achieved a range of 0.2–5.4 ng/g. They also show a comparison of a small number of DP results in sediment using the same samples analysed by high-resolution mass spectrometry (GC/HRMS) with some agreement. However, the authors concluded that the GC/HRMS provided better ultimate sensitivity than their approach.

2.2 New Dechlorane Analogues

Since the DP and dechloranes reviews of 2011, other newly identified related compounds have been reported in the literature [10]. Shen et al. first reported on Chlordene Plus (CP) in sediment of the Great Lakes tributaries [10]. Concentrations were normally observed at low pg/g levels though a higher value was measured in the Niagara River area at 270 pg/g.

Other, new dechlorane analogues have appeared in the literature describing their occurrence in the environment (Fig. 2) [11, 12]. While these appear to be newly discovered chemicals, the analytical methodology remains essentially the same as they are high molecular weight halogenated organic compounds.

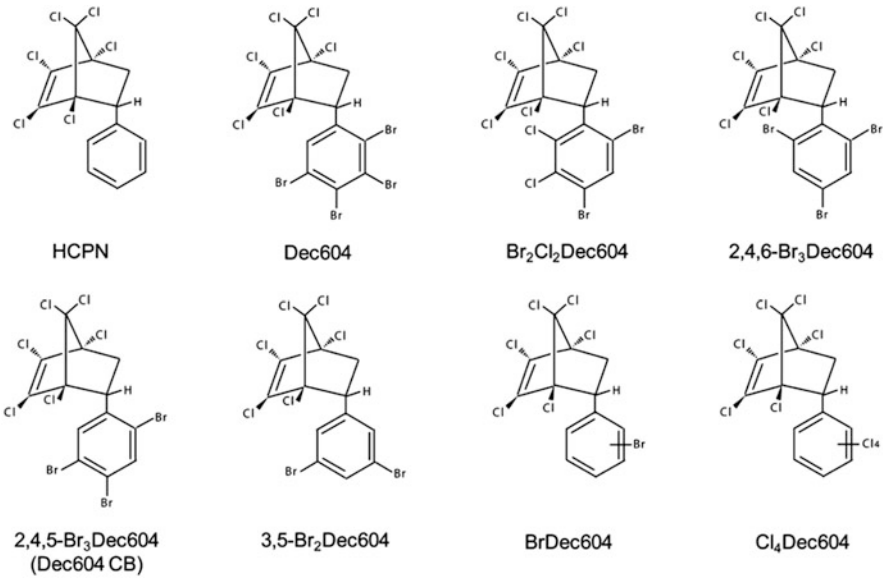

Fig. 2 New compounds identified by Shen et al. [11, 12]

Recent advances in analytical techniques have provided the opportunity for greater efficiency and specificity to measure DP and dechlorane-type compounds in the environment. Caution should be used when analysing DP and dechloranes using GC by monitoring for any in situ dechlorination products. While other new related "DP-like" compounds have been reported on recently, their physical/chemical properties would largely attribute the same analytical considerations as for DP.

3 Chlorinated Paraffins

Chlorinated paraffins (CPs) are complex mixtures of straight-chain *n*-alkanes synthesized by UV-initiated free-radical chlorination of paraffinic feedstocks. Because starting paraffins contain a range of carbon chain lengths (e.g. short, C_{10}–C_{13}; medium, C_{14}–C_{17}; and long, C_{20}–C_{30}) and UV–chlorination is nonselective, resulting technical mixtures are extremely complex. In fact, there can be over 4,000 congeners theoretical present in an individual technical mixture [20].

Short-chain chlorinated paraffins (SCCPs) were first nominated as a candidate POP to the United Nations Stockholm Convention in 2006. To date, voting UN committee members have yet to decide if SCCPs truly fulfils the criteria of a POP as defined by the Convention. Part of the apprehension on the part of the committee is that environmental measurements in remote regions have not been widely demonstrated and, furthermore, it is still unclear at what concentrations negative health effects on biological organisms can be expected. These shortcomings are due, in

part, to a lack of an internationally recognized analytical method that provides the most reliable results [3]. As such, many research groups are investing their efforts in developing standardized methods that can be adopted by the international community.

The inherent complexity of CP mixtures has made their analysis quite challenging. Early methods relied on the separating power of GC coupled to the mass selectively of MS-based detectors. Because of the broad elution profile (>20 min on GC columns) and the large mass range of technical mixtures (*m*/*z* 350–650), interferences from other organochlorine compounds have been widely acknowledged at nominal mass resolving power.

3.1 Analysis of Chlorinated Paraffins

This section will cover recent advances in analysis of CPs with a focus on SCCPs. There have been a number of reviews of CPs in the peer-review literature published prior to 2010. As such, we will present new studies appearing in the literature post-2010. There is also a new review on analytical advances by Van Mourik et al., and unavoidably there is some overlap between that review and what is presented here [21].

Xia et al. applied quantitative structure retention relationships (QSRRs) to predict the gas chromatographic retention times (t_R) of SCCPs [22]. The first step was to identify molecular descriptors that could be used to predict the t_R of SCCPs. Of the 470 possible molecular descriptors which were generated using QSRR software models, 12 were chosen to predict SCCP t_R. The relative importance of each descriptor was then assessed using different multivariate calibration regression methods. The most important descriptor associated with SCCP t_R was found to be the molecular hydrophilic factor. This was not surprising considering that t_R of SCCP congeners of a non-polar GC-analytical column depends on their dispersion forces. The second most important molecular descriptor of SCCP elution was its topological index. This parameter is directly proportional to the number of Cl-atoms which in turn drives the elution order of SCCPs off a GC-analytical column.

The authors then used their model to predict the t_R of 49 C_{10}-CPs. The t_R of 3 of 49 congeners were experimentally measured in the laboratory, and the authors noted that there was a well-defined correlation between t_R empirically with that which was predicted.

Bogdal et al. presented a new approach to analysis of short-, medium- and long-chained CPs [23]. In a single injection, using HPLC/APCI-qTOF-HRMS in the full-scan mode (*m*/*z* 250–1420) at a resolving power of 10,000 and in the absence of an analytical column, the authors were able to detect all three classes of CPs in under 1 min. CP patterns in samples were deconvoluted using a mathematical algorithm. Sample extracts were directly injected into the qTOF-HPLC via flow injections at a flow rate of 150 μL/min. Dichloromethane was purposely added into the ion source

via a T-connection as this created a plasma of Cl ions in the APCI source which enhanced the formation of the $[M+Cl]^-$ adduct ion. The authors noted that these Cl-enhanced ionization conditions suppressed the generation of other ions, and as such detection limits were significantly decreased.

For each CP congener, the 2 most abundant *m*/*z* signals of the $[M+Cl]^-$ isotope cluster were extracted. A total of 522 *m*/*z* values corresponding to CP congener groups with chain lengths of C_{10}–C_{27} and Cl substituents from 5 up to the number of C atoms, i.e. $C_{10}Cl_5$ to $C_{27}Cl_{27}$, were considered in the analyses. The developed method was then applied to quantify CPs in sewage sludge and air samples. In sewage sludge ($n = 7$), the concentration of SCCPs ranged from 135 to 584 ng/g (dw) and between 1,070 and 8,960 ng/g (dw) for MCCPs (Fig. 3). In two urban air samples, concentrations of SCCPs and MCCPs ranged from 1.50 to 3.32 ng/m [3] and 1.32 to 25.9 ng/m [3], respectively.

For sewage sludge samples, APCI gave concentrations of SCCPs that were a factor between 1.4 and 4.4 smaller than those obtained using GC/ECNI-HRMS (Fig. 3). For MCCPs, APCI-based concentrations were greater by a factor between 5 and 16 compared to GC/ECNI-HRMS. There was better agreement between concentrations of CPs measured in air samples using the two methods. An increase

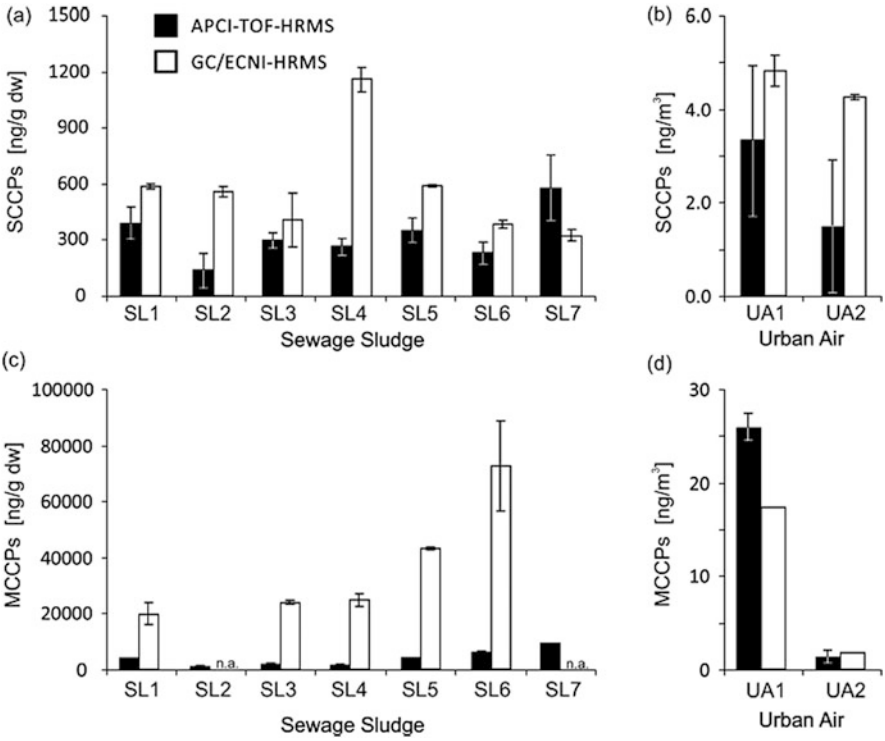

Fig. 3 Concentrations of SCCPs and MCCPs in sewage sludge and urban air samples and comparison of results using APCI-qTOF-HRMS and GC/ECNI-HRMS [23] (with permission)

between 1.5 to 2.8 and 0.7 to 1.3 for SCCPs and MCCPs, respectively, was observed for APCI versus the GC-based method. Because of its short analyses time, the described method is highly amenable as a screening tool for large sets of samples.

Geiss et al. presented a method similar to that of an ISO 120101 for SCCP determination in sediments [24]. The method was based on GC/ECNI-LRMS and on integration of signals of 2 preselected *m*/*z* values (*m*/*z* 375 and 423) over the full retention time range of SCCPs and quantification based upon a multiple linear regression with only these 2 *m*/*z* values. The motivation for this work was to present a simplified method for SCCP analysis in sediment that could be adopted by laboratories within the EU as part of their obligation to measuring these compounds in the European environment.

Sediments were extracted using pressurized liquid extraction (PLE) using *n*-heptane as the extracting solvent. A two-step cleanup procedure was used on the sediment extracts: first, an adsorption chromatography step using activated copper powder (used to remove S-containing compounds) and Al_2O_3. Second, Al_2O_3 clean extracts were then put through a gel permeation chromatography (GPC) using a ClNpahPAE800AC column. With this approach, no interferences from PCBs or MCCPs were observed. Higher Cl containing PCBs, toxaphene and chlorinated naphthalenes were removed by this cleanup procedure. Samples of suspended particulate matter ($n = 28$) collected in Thuringia, Germany, were analysed. SCCPs were detected in nine samples with a maximum concentration of 0.3 ng/g (dw).

Xia et al. provided an extension of the pioneering work of Korytar et al. on two-dimensional comprehensive GC (2D-GC) for analysis of SCCPs [25–28]. In the current study, the authors used μ-ECD to detect SCCPs in fish collected from a river in China. Fish were freeze-dried and SCCPs extracted using PLE with DCM/Hex (50:50). Adsorption chromatography using acidified silica gel was used to remove lipids and some other interfering compounds.

For the GC separations, the 1st column was a non-polar 30 m × 0.25 mm i.d. with a 0.25 μm film thickness DM-1 column, while the 2nd column was 1 m × 0.10 mm i.d. with a 0.1 μm film thickness BPX-50 (50% phenyl polysilphenylene-siloxane). A thermal modulator with cryo-cooling and air heating was used to sorb and desorb analytes. The modulation period was set at 6 s.

Similar to the findings of Korytar et al., the current analytical method was able to separate SCCPs into groups of congeners depending on the C-chain length and the number of Cl-atoms (Fig. 4) [28]. In addition, as illustrated in Fig. 5, SCCPs were also well separated from other OC compounds in the samples analysed. The authors also found that detection limits (DL) using their 2D-GC approach was 3–4 times smaller than using a single GC column. Although to quantitative measurements were made in their study, the authors showed a chromatogram of a fish extract (Fig. 5).

An air sampling campaign in the Asian subcontinent was conducted by Chaemfa et al. to assess the extent of SCCP and MCCP contamination in outdoor air [29]. Polyurethane foam-based passive air samplers (PUF-PAS) were deployed in

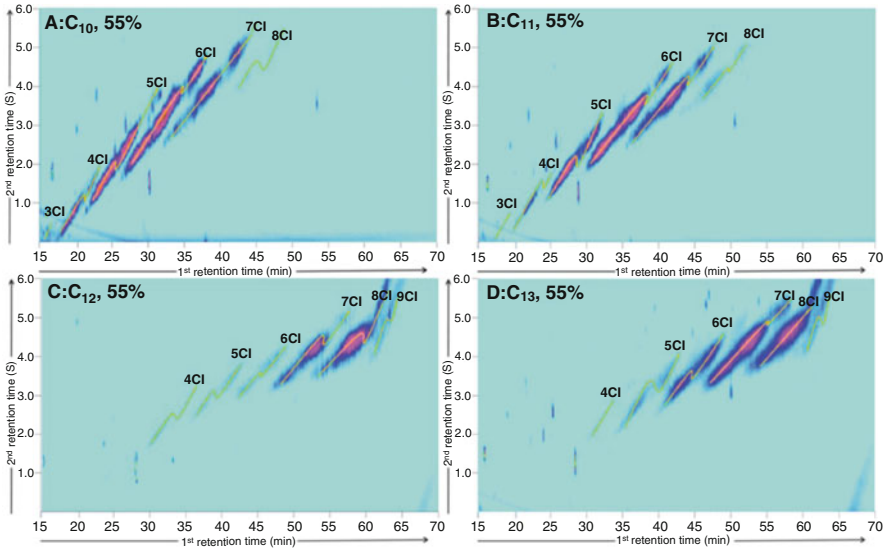

Fig. 4 GC × GC–μECD chromatograms of (**a**) polychlorinated decanes, (**b**) polychlorinated undecanes, (**c**) polychlorinated dodecanes and (**d**) polychlorinated tridecanes, all with a chlorine content of 55% (w/w) [25] (with permission)

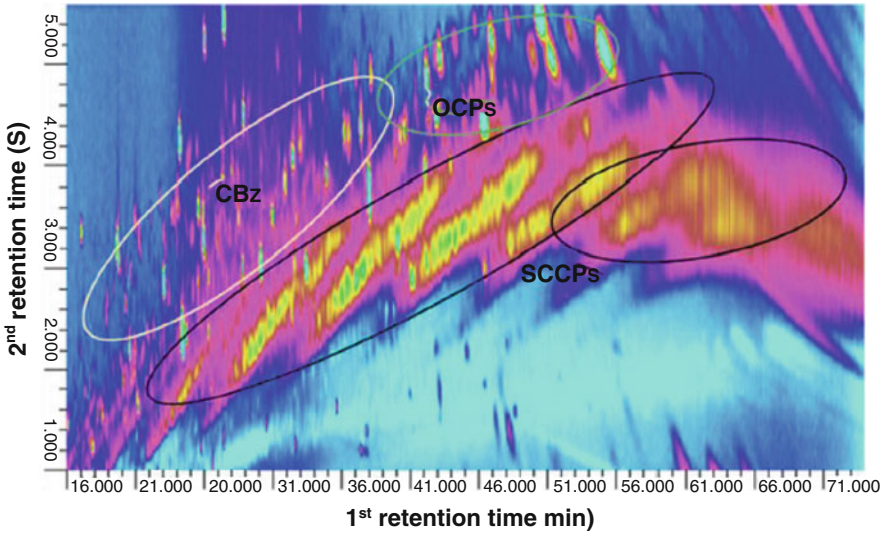

Fig. 5 GC × GC–μECD chromatogram of a fish tissue extract showing the separation of SCCPs from other OC compounds [25] (with permission)

three locations in India (Kolkata, Mumbai and Chennai) during the winter season of 2006 and in industrials and rural areas in Pakistan. PUF samples were Soxhlet extracted using DCM, and extracts were cleaned on a multilayer column containing Na_2SO_4, neutral silica gel and neutral alumina and then finally by a column containing sulphuric acid–silica gel, Florisil and neutral alumina. Analysis and detection of SCCPs and MCCPs were done by GC/ECNI-LRMS on a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The two most abundant isotopes of the $[M\text{-}Cl]^-$ ion cluster for each congener were used for quantification.

Mean concentrations of SCCP and MCCP in samples from India (10.2 and 3.6 ng/m [3]) were greater than those from Pakistan (5.1 and 4.2 ng/m [3]). The rank orders of homologue groups were $C_{10} > C_{11} > C_{12} \approx C_{13}$ and $C_{14} > C_{15} > C_{16} > C_{17}$. Principal component analysis suggested that SCCP and MCCP originated from the same source.

Because of the wide availability of LRMS, Chen et al. invested their efforts on optimizing sample cleanup steps for separating SCCPs from other OC compounds in soils [30]. Four purifications procedures based on adsorption chromatography were tested: (1) silica gel column, (2) alumina column, (3) Florisil column and (4) multilayer silica gel-Florisil composite column. The authors found that a combination of column (1) (i.d. (10 mm), packed from the bottom to the top with 2 g of neutral silica gel (130°C), 5 g of concentrated sulphuric acid-treated silica gel and 2 g of anhydrous Na_2SO_4) and column (4) (i.d. (10 mm), packed from the bottom to the top with 3 g of Florisil, 2 g of neutral silica gel, 5 g of 30% acidified silica gel and 2 g anhydrous Na_2SO_4) gave the most desirable separations.

These columns were used in series to cleanup freeze-dried soil samples collected from a suburban area in Guangzhou, China. Samples were first extracted using Soxhlet with DCM. The dual column adsorption method developed separated 22 PCB congeners, 23 types of OC pesticides, 3 toxaphene congeners and partly *p*,*p*′-DDD and *o*,*p*′-DDD. GC/ECNI-LRMS was used to analyse and detect SCCPs and concentrations ranged from 7 to 541 ng/g with a mean of 84 ng/g (dw). The main SCCP homologues were C_{10} and C_{11} with 6 and 7 Cl-atoms.

Hussy et al. determined the concentrations of SCCPs in marine sediments ($n = 11$) from the Firth of Clyde, Scotland [31]. The authors used a GC/ECNI-LRMS method and compared their measurements to a carbon skeleton GC-FID method described previously by [32, 33]. Freeze-dried sediments were extracted by PLE using iso-hexane, and extracts were cleaned up by adsorption chromatography using Florisil. The concentrations of SCCPs in sediments determined by GC/ECNI-LRMS ranged from 0.4 to 69 μg/kg (dw) and 17 to 379 μg/kg (dw) using the carbon skeleton method. Reasons for the greater concentrations of SCCPs by the carbon skeleton method were unexpected as it was anticipated that some MCCPs would be included in the calculation of SCCP concentrations using GC/ECNI-LRMS, while SCCPs and MCCPs should be easily separated using the carbon skeleton approach. Nevertheless, reported SCCP concentrations were of the same magnitude to total PCBs concentrations previously reported on in the same samples.

Steinberg and Emerson built upon the earlier work of Sistovaris et al. by designing an improved injector-reducing catalyst that performs online dechlorination–

hydrogenation of CPs [34, 35]. The authors observed that earlier in situ dechlorination–hydrogenation based on $PdCl_2$ was not repeatable and often the catalyst deteriorated rapidly. To improve upon this, the catalyst in the injector port was modified by including a sodium borohydride ($NaBH_4$) reduction step. The chemical reduction step resulted in a catalyst that produced quantitative and repeatable dechlorination of CP mixtures. In addition, the lifetime of the new catalyst lasted 3 times longer than the earlier $PdCl_2$ catalyst. No samples were run through the system.

Geiss et al. performed an interlaboratory study on SCCPs in water [36]. There were two types of water samples provided to participants: (1) filtered river water spiked with SCCPs and (2) filtered wastewater spiked with SCCPs. Eighteen laboratories participated in the study and were asked to perform the analysis based on ISO/DIS 12010 analytical method that consisted of a liquid–liquid extraction of the whole water sample, an adsorption chromatography step using Florisil and analysis and detection using GC/ECNI-LRMS monitoring the two ions, m/z 423 and 327, followed by quantification using a multiple linear regression.

The relative reproducibility standard deviation for the two different water samples among the laboratories was between 28 and 34%. This was considered acceptable by the authors considering the multistep cleanup procedure and difficulty in integrating the SCCP unresolved lump.

Gao et al. performed a similar study to Chen et al. where the performance of a variety of adsorbents was evaluated in an attempt to produce extracts that were free of potential interferences [30, 37]. The authors found a Soxhlet extraction of freeze-dried sediment followed by a GPC step and a two-step adsorptive chromatography cleanup method using silica gel (5 g anhydrous Na_2SO_4, 2 g silica gel, 4.5 g acid silica gel and 6 g anhydrous Na_2SO_4), and basic alumina (5 g anhydrous Na_2SO_4, 5 g alumina and 6 g anhydrous Na_2SO_4) produced extracts of sediment that contained undetectable amounts of PCBs, 17 OC pesticides and toxaphene. Sediment extracts from Daliao River, China, ($n = 6$) were analysed by GC/ECNI-LRMS and detection of SCCPs done by monitoring the $[M\text{-}HCl]^{-\cdot}$ ions of each congener. Total SCCPs ranged 54 to 290 ng/g (dw) and the rank order of homologue concentrations was $C_{11} > C_{10} > C_{12} > C_{13}$.

4 Phosphorus Flame Retardants

Phosphorus flame retardants (PFRs) are high-production volume chemicals (HPVCs) touted as replacements for BFRs under regulatory scrutiny, particularly the penta- and octa-formulations of the polybrominated diphenyl ethers (PBDEs); as of 2006 PFRs were reported to comprise 20% of total FR production in Europe [2]. In some cases, PFRs can be used in concert with BFRs, e.g. Firemaster 550, which is comprised of a 60% mixture of PFRs in addition to 40% equal parts of bis(2-ethylhexyl) tetrabromophthalate and tetrabromobenzoate. Production values of PFRs were much greater than those of BFRs or CPs. In addition to their

Table 1 List of selected phosphorus flame retardants (PFRs), their CAS numbers, abbreviations as used in this review and common names

CAS number	Practical abbreviation	Common name
78-40-0	TEP	Triethylphosphate
115-96-8	TCEP	Tris(2-chloroethyl) phosphate
1330-78-5	TCP	Tricresyl phosphate
995-32-4	TEEDP	Tetraethylene diphosphonate
78-42-2	TEHP	Tris(butoxyethyl) phosphate
	TMP	Trimethyl phosphate
791-28-6	TPPO	Triphenylphosphine oxide
513-08-6	TPP	Tripropylphosphate
13674-84-5	TCIPP	Tris(2-chloroisopropyl) phosphate
814-29-9	TBPO	Tributylphosphine oxide
13674-87-8	TDCPP	Tris(2,3-dichloroisopropyl) phosphate
1330-78-5	TMPP	Tris(methylphenyl) phosphate
115-86-6	TPHP	Triphenylphosphate
126-72-7	TDBPP	Tris(2,3-dibromopropyl) phosphate
126-71-6	TIBP	Tris(isobutyl) phosphate
123-73-8	TNBP	Tris(butyl) phosphate
75-51-3	TBOEP	Tris(2-butoxyethyl) phosphate
1330-78-5	TOTP	Tri-*o*-tolyl phosphate
	TMTP	Tri-*m*-tolyl phosphate
	TPTP	Tri-*p*-tolyl phosphate
1241-94-7	EHDPP	2-Ethylhexyl diphenyl phosphate
25653-16-1	T35DMPP	Tris(3,5-dimethylphenyl) phosphate
64532-95-2	T2IPPP	Tris(2-isopropylphenyl) phosphate
1754-47-8	DOPP	Dioctylphenylphosphate
78-42-2	TEHP	Tris(2-ethylhexyl)phosphate
	V6	Tetrekis(2-chlorethyl) dichloroisopentyldiphosphate

Practical abbreviations adopted as per Bergman et al. [100] and van der Veen and de Boer [2]

FR qualities, PFRs have also found application as plasticizers and antifoaming agents in an array of industrial and consumer products including furniture, textiles, building materials and electronics [38].

The PFRs can be designated according to three classifications: inorganic, organic and halogen-containing; for the purposes of this review, only the organic and halogen-containing derivatives will be addressed [2]. The organic PFRs are comprised of phosphinates, phosphonates and phosphate esters. Three examples of halogen-containing PFRs are tris(2-chloroethyl) phosphate (TCEP), tris(chloro-2-propyl) phosphate (TCIPP) and tris(2,3-dichloroisopropyl) phosphate (TDCPP); the first two are among the most widely detected PFRs in the environment with the others including tricresyl phosphate (TCP), triphenyl phosphate (TPHP) and other organophosphate esters [2]. Many of the PFRs are additive FRs, which increases their susceptibility for release into the environment from products during

use or after disposal. A list of some of the most common PFRs for which analytical methods have been developed is shown in Table 1.

Although PFRs have reportedly been in use for over 150 years [2], the first reports of their occurrence, distribution and fate were reported in the 1970s and 1980s that indicated a general lack of environmental stability [39]. However, interest in PFRs re-emerged in the 1990s as a result of reports of their ubiquity in an array of environmental compartments. These reports included the measurement of PFRs in indoor air, water, sediment, indoor air and dust, and fish and other biota [39]. Studies over the past 25 years have also determined the PFRs to be amenable to long-range transport. In terms of toxicological properties, some PFRs are reportedly mutagens, carcinogens, neurotoxins, developmental and reproductive toxins and skin irritants [39]. Howard and Muir determined that seven of top 20 HPVCs with the greatest persistence and bioaccumulation potential were flame retardants; this list included TPP [40].

The review will also focus primarily on developments and trends in methodology since the comprehensive review of environmental occurrence and analysis of PFRs by van der Veen and de Boer [2]. The analysis of these compounds continues to be a challenge given their wide range of physical/chemical properties, e.g. hydrophobicity, and serious issues with blank contamination.

4.1 Sampling, Extraction and Cleanup

As with other compound classes, the goal of the preliminary steps is appropriate sampling of an environmental matrix followed by quantitative extraction of analytes with minimal co-extraction of interfering compounds. Although there are now methods reported for determination of PFRs by direct

injection of extracts, trace analysis of these compounds still requires cleanup, particularly for biota, to achieve good accuracy and precision [41]. In addition, extraction and cleanup steps for analysis of PFRs are trending toward technologies that minimize the volumes of organic solvents used in order to reduce the impact of pervasive blank problems. Blank problems were a significant factor in the considerable variations reported by Brandsma et al. in the first worldwide interlaboratory study [38].

Both Quintana et al. and van der Veen and de Boer published reviews on PFRs that included overviews of sampling and/or extraction in water, air, biota and solid matrices including sediments, indoor dust, air particulate and sewage sludge [2, 42]. One of the critical requirements identified in the previous reviews of analytical methods for PFRs was a paucity of labelled surrogates, particularly for the chlorinated compounds [42]. The availability of both labelled and authentic native compounds is essential for development of accurate and robust methods. Approximately 20 native and 10 deuterated and/or carbon-13-labelled surrogate standards are now commercially available from vendors including Wellington Laboratories, Sigma-Aldrich, Toronto Research Chemicals, Angene Chemical,

AccuStandard, Riedel-de Haën and Fluka. These compounds include d_{12}-tris (2-chloroethyl) phosphate and $^{13}C_{18}$-triphenyl phosphate. Presumably, labelled PFR surrogates will replace those substitutes previously used out of necessity, including the labelled polycyclic aromatic hydrocarbons (PAHs).

4.1.1 Water

Both Quintana et al. and van der Veen and de Boer covered methods for sampling, extraction and cleanup of PFRs for water samples in their reviews of analytical methods for PFRs [2, 42]. van der Veen and de Boer outlined the disadvantages of liquid–liquid extraction (LLE) for extraction of PFRs including the requirement for large organic solvent volumes, foam formation, extraction time, contamination from glassware and difficulties with respect to automating the technique [2]. Both solid-phase extraction (SPE) and solid-phase microextraction (SPME) were investigated in a number of studies to optimize extraction and elution parameters for PFRs. Using SMPE or SPE using an OASIS HLB cartridge, determination of TEHP was problematic. Adsorption of TEHP onto glass and onto dissolved organic matter was reported as potential factors. Based on the studies surveyed, the poly(dimethylsiloxane)-divinylbenzene SPME fibres achieved the best results for extraction of PFRs from water out of the six stationary phases examined [43]. SPME is also reportedly less susceptible to matrix effects.

van der Veen and de Boer presented the extraction efficiencies for different SPME fibres in graphical form; they also identified shortcomings of SPME including the dependence of extraction yield from sample type and the potential need for using a standard addition procedure and sample carry-over effects [2]. A study of SPE phases determined the Bakerbond Hydrophilic DVB cartridges produced the highest recoveries coupled with the fastest extraction times [44]. Common solvents used for elution of PFRs from SPE cartridges included methyl tert-butyl ether (MTBE), ethyl acetate, methanol or a combination of methanol/MTBE.

van der Veen and de Boer referred to only one study that used LLE with toluene for extracting PFRs from water samples. Quintana et al., being an older review paper, identified five studies employing LLE for determination of PFRs in water matrices including wastewater, surface water, drinking water and snow; extractions solvents used were toluene or dichloromethane [42]. They identified the same disadvantages of LLE as van der Veen and de Boer, but also the advantage of the LLE technique's applicability to both filtered and unfiltered samples [2]. In addition, they reported low recoveries for polar PFRs, e.g. TCEP, for LLE of water samples using toluene. Quintana et al. also provided an overview in tabular form of extraction techniques (LLE and SPE), water matrices, extraction conditions, recoveries and limits of quantification (LOQs) [42]. SPE was identified as the preferred method for water samples. Generally satisfactory recoveries for PFRs using a variety of SPE sorbents with the exception of very polar PFRs such as TMP and TEP were reported. The hydrophilic DVB polymeric phases such as OASIS HLB and Bakerbond Hydrophilic DVB provided quantitative extraction yields for even

the more polar compounds; however, the breakthrough volumes were relatively low (0.5 L), compared with less-polar PFRs (2.0 L). Elution solvents for SPE have included MTBE/toluene, acetonitrile, acetone, ethyl acetate and methanol. Quintana et al. also reviewed single examples of the use of membrane-assisted solvent extraction (MASE) and dispersive liquid–liquid microextraction (DLLME) for determination of PFRs in water samples, but these techniques have not since found widespread application [42].

More recent studies of PFRs in surface and drinking waters used SPE more for extraction and preconcentration, rather than for cleanup. Li et al. used a conventional polymerically bonded octadecyl SPE phase (1 g Supelco ENVI-18) for drinking water samples from China ranging in volume from 550 mL to 1 L; cartridges were conditioned sequentially with 10 mL of acetonitrile followed by water [45]. Samples were loaded at 5 mL/min followed by a drying step and elution with two 6 mL aliquots of acetonitrile. Recoveries of most PFRs were satisfactory (70% to 99% with RSDs of 4.7% to 14%); however, recoveries of highly hydrophobic compounds (TEHP and EHDPP) were less than 40%, which the authors attributed to sorption to glass materials. Cristale et al. used a hydrophilic DVB polymeric SPE phase (200 mg OASIS HLB) for 500 mL unfiltered surface water samples; cartridges were sequentially conditioned with 15 mL hexane, DCM, methanol and water [46, 47]. The stationary phase was selected based on its suitability for both relatively polar (PFRs) and apolar (BFRs) analytes. After sample loading, the cartridges were dried under vacuum and eluted with 15 mL 1:1 (v/v) DCM/hexane followed by 15 mL 1:1 (v/v) DCM/acetone. Recoveries for spiked samples were in the range of 70–120% with standard deviations less than 20. A modification of this method was also applied to analysis of PFRs in sediment as discussed later in the chapter.

Quintana et al. identified additional considerations for extraction and cleanup of water samples for PFR, including susceptibility of SPE cartridges to clogging which necessitates pre-filtration and subsequent extraction of the particulate material to ensure quantitative recovery of the most hydrophobic PFRs [42]. Some PFRs, e.g. TMP and TEP, are volatile, and methods based on reduction of solvent extracts prior to instrumental analysis must account for this issue. SPE still appears to be the preferred method for extraction and cleanup for PFRs in aqueous matrices. However, LLE can still find application in comprehensive methods for multiple analyte classes or for matrices that are a challenge for SPE due to the potential for clogging of SPE cartridges or overloading of the sorbent, e.g. wastewater. This is an important consideration given that wastewater treatment plants (WWTPs) can be primary sources of PFRs to surface waters, and as a result wastewater will continue to be a matrix of intense interest [48].

Comprehensive methods for sampling of surface waters and subsequent analyses for multiple compound classes are commonly used by government agencies as part of their long-term research and monitoring programmes. Venier et al. used XAD-2 resin cartridges in concert with a stainless steel submersible pump, a Teflon-lined tubing and a glass fibre filter to remove particles >0.5 μm in order to extract analytes including PCBs, PAHs, organochlorine pesticides, PBDEs and emerging

flame retardants including the organophosphate esters (OPEs) from 100 to 200 L of Great Lakes water [49]. The PFRs included three chlorinated compounds, three alkyl compounds and five aryl compounds. Roughly 340 g of XAD-2 resin was precleaned, packed and spiked with select ^{13}C-labelled PBDEs and PCBs. After sampling, the XAD-2 resin was spiked with recovery surrogates including d_{12}-tris (2-chloroethyl) phosphate and $^{13}C_{18}$-triphenyl phosphate. The resin was then extracted by Soxhlet for 36 h using a 1:1 (v/v) combination of hexane and acetone, after which the extract was reduced by rotary evaporation to roughly 20 mL. Subsequently, the separated water layer was back-extracted with hexane, which was pooled with the original extract. The extract was sequentially fractionated using a 3% (w/w) water-deactivated silica gel column eluted with 25 mL of hexane, 25 mL of 1:1 (v/v) hexane/dichloromethane and 25 mL 2:1 (v/v) acetone/dichloromethane; the PFRs eluted in the third fraction.

4.1.2 Air

Air samples are classified according to two phases; gas phase and particulate phase. The physical/chemical properties of many PFRs result in their being primarily associated with the particulate phase. However, some compounds have relatively high vapour pressures, e.g. TEP, and require application of sampling techniques that account for their significant presence in the gas phase. In a modelling study of flame retardants in both the indoor and outdoor environments, Liagkouridis et al. determined that a significant number of PFRs could partition into the gas phase [50].

Quintana et al. reviewed both active and passive air sampling methods for PFRs with emphasis on the particulate phase under low sampling rates (1–15 L/min) and moderate sampling volumes (0.1–14 m [3]) [52]. Active sampling media included glass- and quartz-fibre filters and SPE cartridges. The use of polyurethane foam (PUF) devices and SPE membranes was discouraged due to the requirement for greater extraction solvent volumes that exacerbates the issue of blank contamination. In addition, the use of SPE enables a rinse step to reduce co-extractive interferences prior to elution of PFRs. However, SPE is reportedly susceptible to high back pressures when pumping air, compared to PUFs and SPE membranes [42]. Some studies also described automation of the PFR desorption step using stainless steel cells and switching valves. A summary of active sampling and desorption conditions was presented in tabular form; in addition to glass- or quartz-fibre filters, sorbents included charcoal, aminopropyl silica, styrene–divinylbenzene and PUF. Desorption solvents included toluene, MTBE, dichloromethane, acetone, methanol and a mixture of hexane and MTBE. Limits of detection (LODs) for PFRs in indoor air were generally in the low ng/m [3] range; LODs varied depending on whether the methodology was based on online or off-line desorption. Alternative sampling devices were based on SPME, but the capacity associated with this technique was very low compared to SPE. Only one reference was cited based on passive sampling.

van der Veen and de Boer identified a broader range of sampling and extraction techniques for air in their more recent review, including passive flux sampling, SPE membranes, SPME, PUFs, glass fibre filters and glass fibre filters with a supplemental cellulose filter [2]. Desorption solvents included dichloromethane, methanol, MTBE and acetone. There was increased emphasis on the use of ultrasonication as an extraction method due to its requirement for lower solvent volumes, rapid extraction time and quantitative extraction of TBOEP, compared to Soxhlet extraction. van der Veen and de Boer also identified the issue of the potential for high back pressure when using SPE cartridges, but also indicated that methodology based on smaller SPE cartridges containing 10–25 mg of sorbent could be viable for PFRs. As noted by Quintana et al., use of SPE provides potential for incorporation of a rinse step into the method [42]. In a 2005 study conducted by Staaf and Ostman, four different stationary phases were investigated for their applicability for the determination of PFRs in air samplers; the use of an aminopropyl silica sorbent in concert with elution using MTBE produced the best results [51]. As with water as a matrix, the determination of TEP requires special consideration; recovery of this compound is roughly fivefold higher using SPE, compared with glass fibre filters. Overall, van der Veen and de Boer recommended SPE for the determination of PFRs in air rather than glass fibre filters, except for TDCPP [2].

Castro-Jiménez et al. studied the occurrence of PFRs in the atmospheres of the Mediterranean and Black Seas [52]. Air was sampled using high-volume samplers containing both polyurethane foam (PUF) and quartz-fibre filters (QFFs). The PUF samples were all below the method limits of detection; only the particulate samples from the QFFs were reported. The QFFs were extracted by Soxhlet in 2:1 (v/v) DCM methanol for 24 h and subjected to a cleanup procedure based on 3% water-deactivated alumina (3 g) with a top layer of anhydrous sodium sulphate. Three fractions were collected sequentially by elution using 5 mL hexane, 12 mL 2:1 (v/v) DCM/hexane and 12 mL of DCM; the second and third fractions contained the PFRs. Möller et al. sampled the atmosphere of the North Sea to study the long-range transport of PFRs using a high-volume air sampler; PUF/XAD-2 resin columns and glass fibre filters (GFFs) were extracted separately for gaseous PFRs and particulate-bound PFRs, respectively [53]. Both sample components were extracted by Soxhlet using DCM for 16 h. Extracts were reduced in volume by rotary evaporation and subjected to an open-column cleanup using 2.5 g of 10% water-deactivated silica with a layer of 3 g of anhydrous sodium sulphate. The column was eluted using 20 mL of hexane to afford a non-polar fraction that did not contain any PFRs; analytes were eluted in the second fraction by addition of 30 mL 1:1 (v/v) DCM/acetone. Internal standard recoveries were $131 \pm 46\%$ and $176 \pm 40\%$ for filter samples and $241 \pm 130\%$ and $224 \pm 15\%$ for $[D_{27}]$-TNBP and $[D_{15}]$-TPHP, respectively; the authors did not provide any assessment of these high recoveries. Surrogate recovery-corrected values for the analytes ranged from $77 \pm 5\%$ for TCEP to $111 \pm 4\%$ for TEHP with a mean overall value for all the PFRs of $91 \pm 13\%$. Möller et al. used the same methodology to study the occurrence and distribution of PFRs and plasticizers in the atmosphere over the northern Pacific

and Indian Oceans [6]. Internal standard recoveries for the particulate phase were $73 \pm 24\%$ and $89 \pm 23\%$ for [D_{27}]-TNBP and [D_{15}]-TPHP, respectively. Cheng et al. used a very simple sample preparation procedure for GFFs from a high-volume air sampler as part of a study of PFRs in the atmosphere of the West Pacific, Indian Ocean and Southern Ocean [54]. The GFFs were cut into 10 pieces of 1.5 cm diameter each and extracted by ultrasonication in 5 mL water and then centrifuged. No QA/QC information was provided with respect to analyte recoveries.

Salamova et al. measured PFRs in the Great Lakes atmosphere using methodology developed for the Integrated Atmospheric Deposition Network (IADN) [47]. The IADN protocol provides a very detailed description of a comprehensive method for extraction and cleanup of air and precipitation samples; the protocol includes 84 PCBs, 22 organochlorine pesticides (OCs), 16 PAHs, 47 FRs (including 36 PBDEs) and 12 PFRs [55]. As per other studies, the authors reported particle-phase PFRs accounted for $95 \pm 2\%$ of the total of the vapour and particle phases. Only four PFRs were detected in the vapour phase (TNBP, TCEP, TDCPP and TPP) at percentages of total concentrations ranging from 3.4% (TCIPP) to 10.2% (TPP). The QFFs from high-volume air samples (24-h samples representing ~820 m [3] collected every 12 days) were Soxhlet extracted for 24 h in 1:1 (v/v) acetone/hexane. The extract was reduced in volume by rotary evaporation, exchanged with hexane and subjected to an open-column cleanup step using 3.5% (w/w) water-deactivated silica gel. In the elution with 25 mL of hexane, 25 mL 1:1 (v/v) hexane/DCM and 25 mL 7:3 (v/v) acetone/DCM, the PFRs eluted in the third fraction.

4.1.3 Solid Matrices Including Sludge, Dust and Sediment

Quintana et al. reviewed sample preparation methodologies for a number of solid matrices including indoor dust, sludge, sediment, urban dust and air particulate [42]. For the most part, a cleanup procedure was required subsequent to extraction to prepare extracts for instrumental analyses. For solid matrices, medium-polarity solvents including ethyl acetate, dichloromethane and acetone were commonly used, mostly in conjunction with sonication, Soxhlet and pressurized liquid extraction (PLE) which now is also commonly referred to as accelerated solvent extraction (ASE). Cleanup methods included SPE, LLE, gel permeation chromatography (GPC) in concert with SPE and the use of a sorbent (alumina) in concert with PLE extraction of urban dust using ethyl acetate that afforded a combined extraction and cleanup procedure. Extracts from highly complex matrices with high percentages of organic carbon, e.g. sewage sludge, required a GPC procedure in addition to SPE. Although GPC continues to be cumbersome and time-consuming for laboratories that lack automated equipment, the method in combination with ancillary procedures such as SPE also affords extracts from difficult matrices such as sludge and biota that are amenable to a wide variety of analytical techniques. In their own study, Quintana et al. reported selective extraction of PFRs from urban dust based on use of mild extraction conditions and online purification of the extract using PLE

with ethyl acetate at 50°C [56]. A layer of alumina in the extraction cell retained polar interfering compounds.

As with the earlier review by Quintana et al., extraction techniques for PFRs in solid matrices, particularly sediment, as reviewed by van der Veen and de Boer included ultrasonication and shaking. Solvents used for extraction of PFRs, in whole in combinations, included acetone, acetonitrile, ethyl acetate, dichloromethane, MTBE [2, 42]. The study of PFRs in sediments from rivers in Spain and the United States by Garcia-López et al. received particular attention; a variety of solvents were assessed using microwave-assisted extraction (MAE) with acetone followed by acetonitrile providing the best results [57]. The limited time required for the procedure and small solvent volumes were emphasized as advantages over other techniques. Cleanup techniques included open-column silica or Florisil, SPE and GPC. Leonards et al. extracted PFRs from sediments from Norway using a 1:1 mixture of dichloromethane and acetone using ASE followed by a cleanup method based on GPC; the fraction containing aliphatic PFRs was further purified using SPE [58].

Recent studies on the occurrence of PFRs in indoor and outdoor dust samples from China, Norway, the United States and New Zealand were based on the methodology developed by Van den Eede et al. and based on ultrasonic extraction and a two-stage SPE cleanup; this method was optimized for determination of a number of different FR classes including PBDEs, hexabromocyclododecane (HBCD), BFRs and PFRs (Fig. 6) [59–62]. Dust samples (~75 g) were extracted in 2 mL 3:1 (v/v) hexane/acetone by a combination of vortexing and ultrasonication (2 cycles of vortexing for 1 min followed by ultrasonic extraction for 5 min); this extraction procedure was repeated three times. After each extraction cycle, the extracts were centrifuged, affording a pooled centrifuged extract that was taken to dryness under nitrogen and redissolved in hexane for Florisil SPE cleanup. The SPE cartridges were prewashed with 6 mL of hexane, after which the sample extract was loaded and eluted into two fractions using 8 mL of hexane and 10 mL of ethyl acetate, respectively. The first fraction contained the PBDEs and some of the other BFRs including HBCDs, while the second fraction contained the PFRs and some HBCDs. The first fraction was subjected to an additional SPE cleanup using 44% acidified silica that was prewashed with 6 mL of hexane; analytes were eluted using 10 mL 1:1 (v/v) hexane/DCM. Typical recoveries for PFRs based on spiking studies ranged from 80 to 110% with inter-day RSDs ranging from 11 to 24% depending on the spiking concentration. Percentage accuracy for PFRs based on Standard Reference Material (SRM) 2585 (indoor dust) ranged from 97 to 157%.

Methods developed by Cristale et al. were applied to multiple compound classes, including PFRs, in water and sediment, and in sediment, sludge and dust [46, 47]. The procedure for extraction and cleanup of PFRs in surface water was discussed previously; freeze-dried sediment samples were extracted in 20 mL of 5:2 (v/v) ethyl acetate/cyclohexane by vortexing for 1 min followed by ultrasonication for 10 min [55]. This procedure was repeated for three cycles. Following reduction of the extract under nitrogen and addition of copper to remove sulphur, a cleanup step was performed using 10 g Florisil SPE cartridges per-conditioned using 60 mL

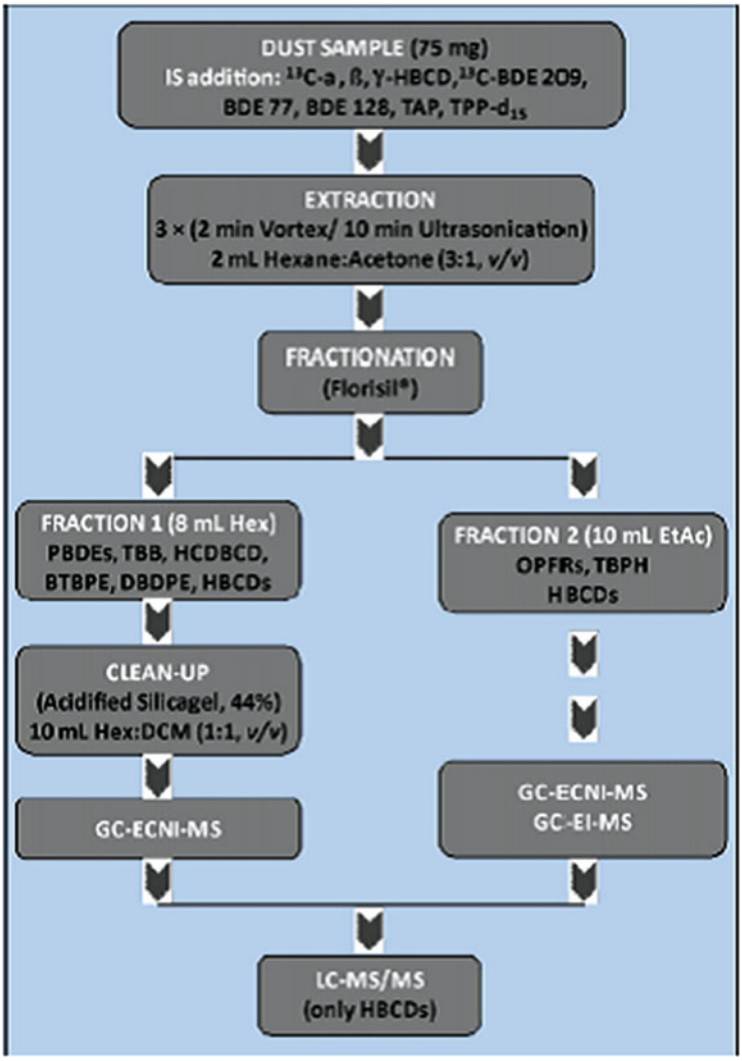

Fig. 6 Methodology developed by Van den Eede et al. and based on ultrasonic extraction and a two-stage SPE cleanup for dust samples [63] (with permission)

of 5:2 (v/v) ethyl acetate/cyclohexane. After sample loading, the PFRs were eluted using 60 mL of 5:2 (v/v) ethyl acetate/cyclohexane. The volumes of solvent used in this study were considerable, which could exacerbate blank problems. The method for determination of PFRs, BFRs in sediment, sludge and dust was based on the same protocol as that for sediment previously described, but with varying amounts of sample, surrogates, solvent and Florisil [46].

Both Ballesteros-Gómez et al. and Brandsma et al. used direct injection of dust extracts to study the influence of electronic equipment on levels of PFRs

[64, 65]. Ballesteros- Gómez et al. used shaking for 24 h in DCM followed by ultrasonication for 10 min to extract resorcinol bis-(diphenylphosphate) (PBDPP) and bisphenol A bis(diphenylphosphate) (BPA-BDPP) from ~50 mg samples of plastic products including powerboards and televisions [64]. The DCM extracts were diluted 10–100 fold with methanol and then centrifuged to precipitate solids prior to analysis. Brandsma et al. used a two-stage extraction procedure based on 1 min of vortexing followed by 15 min of ultrasonication; the extraction procedure was first carried out in acetone, followed by toluene [65].

4.1.4 Biological Matrices

As of 2012, the amount of information on methodology for the determination of PFRs in biota was far less than for the abiotic matrices; the review by van der Veen and de Boer provided five examples for biota [2]. The biota samples were prepared using homogenization and freeze-drying with/without subsequent homogenization. Extraction methods included shaking and ASE; extraction solvents included acetone/MTBE, MTBE, 1:1 dichloromethane/acetone and ethyl acetate/cyclohexane (5:2) and cyclohexane/diethyl ether (3:1). A report by Campone et al. described the use of matrix solid-phase dispersion using Florisil and alumina for extraction and cleanup of PFRs [66].

In the past 5 years, there has been heightened interest in the determination of PFRs in biological matrices including human breast milk, urine and plasma. Compounds of interest include not only the parent triester flame retardants but also the diester phosphoric acid metabolites, due to the reported rapid and strong susceptibility of the triester compounds to metabolic processes [67]. In their review of analytical methods for emerging contaminants in human matrices, Dirtu et al. provided an overview of typical analytical methods for determination of organophosphate triesters and their associated metabolites (dialkyl and monoalkyl phosphates) [67]. The authors also emphasized the susceptibility of the parent compounds and metabolites to oxidation and hydrolysis at low pH; both of which are factors that complicate selective extraction from biological matrices.

Dirtu et al. classified human urine as an “advantageous” matrix because of its low lipid and protein content and the availability of large sample volumes that could compensate for low concentrations of metabolites; however, the matrix can contain large numbers of interfering compounds, and the metabolites themselves can be highly acidic which makes protonation prior to extraction difficult [67]. The major metabolite of TPHP, diphenyl phosphate (DPHP), is among the most analysed metabolites in human urine. Typical sample preparation of urine for analysis of PFR triesters and their associated metabolites involves adjustment of pH within a range of <1 to 6.5 depending on the compounds of interest, using buffers, hydrochloric acid or acetic acid. Liquid–liquid extraction, SPE and matrix solid-phase dispersive (MSPD) extraction have been applied to extraction of the triesters and their metabolites from urine.

The method developed by Schindler et al. for a range of dialkyl metabolites based on hydrophilic–lipophilic polymers for extraction yielded very good recoveries; SPE using a cross-linked styrene–divinylbenzene copolymer afforded extracts that were subjected to a derivatization step using an alkylating agent (pentafluorobenzyl bromide) and an ancillary cleanup using Florisil and PSA SPE (ethylenediamine-*N*-propyl phase that contains both primary and secondary amines and is a weak anion exchanger) [68, 69]. The PSA phase is similar to aminopropyl phases in terms of selectivity, but has higher capacity due to the presence of secondary amine and has a strong affinity and high capacity for removing fatty acids, organic acids and some polar pigments and sugar; it has also been demonstrated to reduce matrix enhancement effects encountered during GC analysis of food products. Dirtu et al. emphasized the good accuracy, precision and sensitivity of this method but indicated it was time-intensive; the use of deuterated internal standards as method recovery spikes was also highlighted [67].

Cooper et al. developed a method for determination of bis(2,3-chloroisopropyl) phosphate (BDCPP) and DPP, the primary metabolites of TDCPP and TPP, respectively, in urine [70]. The authors evaluated a range of SPE phases and determined weak anion exchange to be the most effective mechanism. Urine (5 mL) was spiked with deuterium-labelled surrogates and diluted 1:1 with water and pH adjusted to 6.5 using 0.1 M acetic acid if required. The SPE cartridge (StrataX-AW, Phenomenex) was conditioned with methanol followed by water and then sample loading at a flow rate of 1 mL min^{-1}. The cartridge was subsequently washed with 2 mL of acetonitrile, dried under vacuum and then eluted with 2 mL of acetonitrile containing 5% pyrrolidine, taken to dryness under nitrogen and then reconstituted in water/methanol (4:1) and filtered (0.2 μm) in preparation for LC tandem MS analysis. In a related but more recent and comprehensive study, Van den Eede et al. determined that a comprehensive method for a wide range of PFR metabolites would be difficult to achieve given their range of chemical and physical properties, e.g. hydrophobicity and ionization potentials [71]. As a result, they developed two different SPE/derivatization procedures for determination of six PFR dialkyl and diaryl phosphate metabolites. This study also served as a comparison of the method with that developed previously by Schindler et al. as described previously [68, 69]. While the weak anion exchange sorbent (StrataX-AW, Phenomenex) used by Cooper et al. resulted in satisfactory recoveries, matrix effects were three- to ninefold greater in terms of signal suppression with this phase, compared to Oasis WAX (Waters) [70]. However, the Oasis WAX phase also resulted in high blanks for DPP, which according to the authors is sometimes used as a plasticizer. This blank issue persisted even after washing with 5% NH_4OH in methanol. The authors selected the Oasis WAX phase for optimization of their method based on its suitability for the other five analytes of interest. Urine was pretreated by adjustment to pH 5 using a sodium acetate buffer and then spiked with labelled surrogates. The SPE cartridges were preconditioned using 1 mL basic methanol followed by 2 mL of water (pH 5) followed by loading of 2 mL of urine sample followed by a wash step using 2 mL of 30% methanol in water (pH 5) and subsequent elution in 5 mL basic methanol. For two metabolites that exhibited low sensitivity and weak

retention in RPLC (bis(2-chloroethyl) phosphate and bis(1-chloro-2-propyl) phosphate), a derivatization step based on alkylation with trimethylsilyldiazomethane (TMSDM) was required. Based on LC tandem MS analysis, recoveries for urine at concentrations of 3 ng/mL ranged from 69 to 119% with inter-day imprecision of less than 31%.

Reemtsma et al. determined 14 monoalkyl phosphates (MAPs), dialkyl phosphates (DAPs) and dialkyl thiophosphates as metabolites of PFRs in urine [72]. The samples (3 mL) were extracted at their natural pH by mixing with acetonitrile (3 mL) and shaking and then centrifuged for 10 min at 3,800 rpm. The supernatant was reduced in volume, water was added and the extract filtered through a 0.45 μm cellulose acetate filter; this represented the extent of the cleanup procedure. The most recent methodology for PFR urinary metabolites developed by Van den Eede et al. was based on SPE using a polymeric weak anion exchange sorbent preconditioned with 2 mL acetonitrile followed by 2 mL of water [71, 73]. After sample loading, the cartridge was rinsed with 2 mL water and then eluted with 2 mL 5% triethyl amine in acetonitrile.

Dirtu et al. also reviewed a small number of studies describing methods for determination of PFRs in human plasma and breast milk [67]. In most cases, a protein denaturation step using formic acid or a combination of methanol and acetonitrile was used for plasma sample pretreatment. Extraction methods for plasma included LLE, SPME and SPE. The LLE methods suffered from low recoveries of the more polar compounds including TEP and TCEP. Use of SPME (methylsiloxane–divinylbenzene) afforded cleaner extracts, but similar to LLE recoveries were low. For both the LLE- and SPME-based techniques, sensitivity and reproducibility were satisfactory. Amini and Crescenzi developed an online SPE (C_{18}) method for eight PFR triesters; plasma was combined with formic acid for the sample loading followed by elution of the analytes as part of the chromatographic programme [74].

Breast milk is a matrix high in both protein and lipids. For breast milk, only one study by Sundkvist et al. was cited by Dirtu et al. for eight PFRs including TDCPP and TNBP (measured at 45 ng g^{-1} and 12 ng g^{-1} lipid, respectively) [67, 75]. Milk samples (50 g to 100 g) were pretreated by mixing with ethanol and sodium oxalate in methanol. Extraction was based on three consecutive LLE cycles (150 mL 7:10 v/v diethyl ether/hexane). For the cleanup, 3 cycles of LLE using 10 mL hexane/acetonitrile (1:1 v/v) per g of lipids and acetonitrile with H_3PO_4 buffer with 5 mL MTBE were followed by GPC for lipid removal using Bio-Beads (S-X3, 30 g) and elution with 3:1 v/v cyclohexane/ethyl acetate. More recently, Kim et al. modified the method of Sundkvist et al. for determination of 10 PFRs in breast milk from Asian countries [75, 76]. Ten mL samples of breast milk were freeze-dried, homogenized with sodium sulphate, spiked with surrogates and extracted in hexane/acetone (1:1 v/v) using PLE. Cleanup was based on GPC using Bio-Beads and elution with cyclohexane/ethyl acetate (3:1). An ancillary open-column cleanup was performed on 5% water-deactivated silica conditioned with hexane followed by sample loading and elution with DCM.

The determination of PFRs and their metabolites in lipid-rich biological samples remains a daunting challenge, particularly when LC/MS is used. Despite the excellent ESI response factors for PFRs, matrix effects have hampered development of accurate and precise methodology [41]. Chen et al. used ASE extraction in 1:1 (v/v) DCM/hexane of herring gull egg homogenates followed by cleanup using aminopropyl silica SPE cartridges for determination of PFRs [77]. The SPE column was prewashed with 15 mL 1:1 (v/v) DCM/methanol followed by 15 mL DCM and 20 mL hexane for conditioning. An initial fraction was eluted in 2 mL 1:4 (v/v) and discarded, followed by elution of the PFR fraction using 4 mL 1:4 (v/v) DCM/hexane. Mean PFR recoveries ranged from 67 to 104% with minimal matrix effects observed for LC tandem MS assessed using standard addition. In order to reduce required organic solvent volumes, Chu and Letcher recently developed a method for trace levels of PFRs in lipid-rich biological samples based on extraction by ultrasonication and cleanup by dispersive SPE using a PSA sorbent [52]. One-gram samples of egg or animal tissues were extracted three times (10 min per cycle) in 4 mL of 1:1 (v/v) DCM/hexane; sodium chloride and magnesium sulphate were added and the sample vortexed prior to extraction. A 300 mg portion of PSA sorbent was then added to the sample extract. The difference between SPE and dispersive SPE is straightforward in that the sorbent is added to the extract as a dispersed agent, rather than being contained in a cartridge, which reportedly simplifies the cleanup process and reduces solvent consumption [41]. Recoveries for PFRs ranged from 54 to 113% with an RSD $<17\%$.

Brandsma et al. developed a sample extraction and cleanup procedure applicable to PFRs in both sediment and biota [78]. Samples were freeze-dried, homogenized and extracted with 1:1 (v/v) DCM/acetone using PLE. Cleanup was performed using 500 mg NH_2 SPE cartridges washed and conditioned with 10 mL DCM followed by 6 mL hexane. After sample loading, the cartridge was rinsed with 6 mL to remove interferences followed by elution of PFRs using 4 mL of 1:4 (v/v) DCM/hexane followed by 15 mL of DCM. For sediment samples, an additional fraction was eluted using 10 mL of acetone.

Su et al. have recently published quite extensively on the determination of PFRs and their metabolites in avian plasma, which has extended the scope of methods for complex biological matrices beyond that of human urine [79–81]. For example, the method of Su et al. is also applicable to bovine serum, chicken eggs and pork liver (Fig. 7) [80]. The authors used a novel approach based on ASE to selectively and sequentially extract the parent triester PFRs followed by the diester metabolites. Extraction in 1:1 (v/v) DCM/hexane yielded the triester fraction, while subsequent extraction in 1:1 (v/v) hexane/acetone with 1% acetic acid afforded a diester metabolite fraction. The diester fraction was subjected to a cleanup step based on aminopropyl silica SPE. Cartridges were prewashed with 10 mL of 5:95 (v/v) water/methanol with 0.1 molar ammonium acetate followed by cleaning and conditioning with 3 mL each of methanol and acetone. After sample loading, the cartridge was rinsed sequentially with acetone (6 mL), methanol (3 mL), 5:95 water/methanol (1.5 mL containing 0.005 molar ammonium acetate) and 5:95 water/methanol (1.5 mL containing 0.1 molar ammonium acetate). The PFR

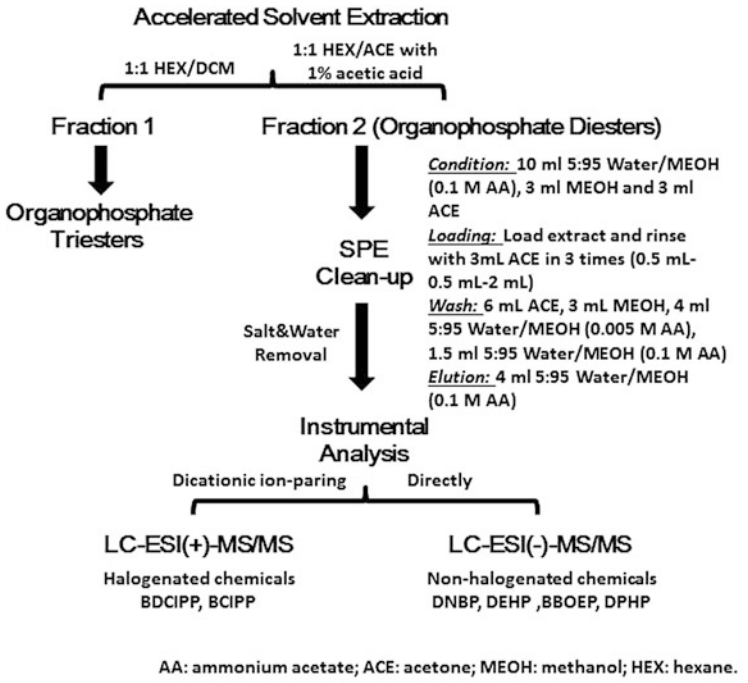

Fig. 7 Sample pretreatment and instrumental analysis of PFRs and metabolites in biological matrices [80] (with permission)

diesters were then eluted with 4 mL 5:95 (v/v) water/methanol containing 0.1 molar ammonium acetate. Recovery efficiencies ranged from 55 to 116%.

4.2 Instrumental Analysis

Up until the last 5 years, many methods for PFRs were based on GC/MS or GC with nitrogen–phosphorus detection (GC/NPD). In addition, many analysts were forced to employ external calibration methods for quantification due to the paucity of authentic labelled surrogates. Ultimately, the number of commercially available labelled surrogates will increase which will enable the use of isotopic dilution for most PFRs. Many methods now exploit the resolving power, selectivities and sensitivities of the newest generations of LC/MS technology; GC/MS and LC/MS are now the preferred techniques for instrumental analysis of PFRs based on analytical methods reported in the literature over the last four years. van der Veen and de Boer concluded that GC/ICP/MS appeared to be the most promising method for instrumental analysis of PFRs due to the reduced complexity of the chromatograms and comparable sensitivity to GC/NPD and other GC/MS techniques; however, to date GC/ICP/MS has not found widespread application [2]. The most

recently reported methods use LC/MS, GC/MS, multiple LC/MS runs under different ionization conditions, or a combination of LC/MS and GC/MS. Analytical methods involving multiple techniques are primarily the result of the requirement for analysis of a suite of PFR analytes with a broad range of chemical/physical properties.

Currently, the chemical/physical properties of the PFRs and their metabolites are the determining factor in terms of selection of instrumentation, i.e. GC/MS vs. LC/MS. Techniques based on LC/MS were previously more susceptible to matrix effects (MEs), compared to GC/MS and GC/NPD, but recent advances in cleanup methodology and the increased commercial availability of surrogate-labelled standards have improved the accuracy of LC/MS for analysis of PFRs [67]. For the purposes of this review, MEs are presented on a percentage basis as [100 – ME (%)] as defined by Gosetti et al.; ME values >100% indicate signal enhancement, while ME values <100 show signal suppression [82]. The work of Gosetti et al. is an excellent primer on many aspects of signal suppression and signal enhancement in LC tandem MS [82].

4.2.1 Water

In the review of methods for PFR analysis by van der Veen and de Boer, there is more of a balance between the use of GC and LC techniques; of the nine studies covered, six were based on GC and the remaining three on LC [2]. All three of the LC methods were based on tandem MS detection, while GC detection methods were based on NPD or MS (EI mode) detection. Typical GC separation parameters included a DB-5 (equivalent) stationary phase (30 m length × 0.25 mm i.d. × 0.25 μm stationary phase thickness) using splitless or programmable temperature vaporization (PTV) in splitless mode. The LC stationary phases ranged from C8 to C12 to C18 (15–25 cm column length × 2.1 mm – 4.6 mm i.d. × 4 μm to 5 μm particle sizes). Martínez-Carballo et al. used a C8 analytical column (15 cm length × 2 mm × 5 μm particle size) in conjunction with a water/methanol mobile phase (injection volume of 10 μL) with each solvent modified with 0.1% (v/v) formic acid and 10 mM ammonium acetate for separation of 9 PFRs [49]. The triple-quadrupole mass spectrometer was operated in positive ion EI, and quantification was by multiple reaction monitoring (MRM); the authors presented the MRM transitions in tabular form. Comparison of LOQs for methods based upon GC/NPD (5–10 ng mL^{-1}), GC/MS (3–30 ng mL^{-1}) and LC/MS/MS (0.5–13 ng mL^{-1}) appeared to compare favourably, and in general percent recoveries and percent RSDs were satisfactory for all the reported methods.

García-López et al. used LC/MS/MS for determination of PFRs and their metabolites in water samples [83]. The LC separation was based on a C18 column (10 μL injection on a 10 cm column length × 2 mm i.d. × 3 μm particle size) with a water/methanol mobile phase modified with 5 mM ammonium acetate. Interestingly, the parent triester compounds were determined using positive ESI, while the diester metabolites were determined in negative ESI mode; these analyses were

achieved using separate runs. The MS/MS parameters were optimized according to capillary voltage, MRM transitions and collision energies; these parameters were presented in tabular form. The authors also presented the proposed fragmentation pathways for TPP in positive ESI mode. More recently, Li et al. used a UPLC/MS/MS method for determination of PFRs in drinking water from China [45]. The UPLC method used a 10 cm column length × 2.1 mm i.d. × 1.7 μm particle size phenyl column with a water/methanol mobile phase; each solvent was modified with 0.1% formic acid (v/v). The MS was operated in positive ESI mode and MRM. The method LODs ranged from 0.50 ng L^{-1} to 1.00 ng L^{-1}. Venier et al. used the International Atmospheric Deposition Network (IADN) GC/EI/MS method for determination of PFRs in Great Lakes surface waters; the analyte suite included three chlorinated compounds, three alkyl compounds and five aryl PFRs [49].

4.2.2 Air

In their review of PFRs, van der Veen and de Boer provided an overview of ten studies that reported the determination of PFRs in air [2]. Half the studies employed GC/NPD or GC with flame photometric detection (FPD). In terms of GC separation conditions, the vast majority of studies employed non-polar DB-1 equivalent or DB-5 columns (30 m length × 0.25 mm i.d. × 0.10 μm or 0.25 μm stationary phase thicknesses) using split and/or splitless injection. The study by Björklund et al. was of particular note as it provided comparative data for PFRs using GC/NPD, GC/MS (ion trap using collision-induced dissociation (CID) and selected ion monitoring (SIM) in EI mode and CID and selected reaction monitoring (SRM) in positive ion chemical ionization (PICI) mode) using the same chromatographic parameters [84]. The GC/MS PICI technique was selected based on the observation of less fragmentation of PFRs, but van der Veen and de Boer noted some peak tailing for higher polarity PFRs due to the non-polar nature of the DB-5 stationary phase; a more polar stationary phase was recommended [2]. In addition, the LOD for the PICI method was roughly 50-fold lower (0.1–1.4 ng m^{-3}) than for the EI technique; IDLs for the NPD technique were consistently below 5 pg, which was better than those achieved for some PFRs using the PICI, but with a corresponding potential lack of selectivity. Only one example based on LC/MS (triple quadrupole equipped with ESI) was provided; a C8 stationary phase was determined to be superior to a C18 phase when used in conjunction with a methanol/water mobile phase modified with 1 mM trifluoroacetic acid (TFA) [85]. The resulting method LODs ranged from 0.4 to 19 ng m^{-3} which compared favourably with those of the PICI technique of Björklund et al. [84].

Many contemporary research and monitoring programmes continue to use GC/MS for analysis of PFRs in air samples. In their studies of PFRs in air from the Great Lakes and European Arctic, Salamova used GC/MS analysis in positive EI mode using SIM according to the IADN protocol (2013) for analysis of PFRs using GC/MS in EI mode [47, 55, 86]. Separation of PFRs was achieved using a DB-5MS column (2 μL injection on a 30 m column length × 0.25 mm i.d. × 0.25 μm

film thickness) with helium as the carrier gas at 1.5 mL min^{-1}. Average surrogate recoveries were in the range of 70% to 100%. Cheng et al. used LC/MS/MS in positive ESI mode for analysis of PFRs in air from the West Pacific and Indian Oceans [54]. A C18 column (15 cm column length × 4.6 mm i.d. × 5 μm particle size) was used in conjunction with a water/methanol mobile phase modified with 0.1% formic acid. The method LOQs were 0.1, 0.6, 0.06 and 0.5 pg m^{-3} for TBOEP, TCEP, TCIPP and TDCPP, respectively.

Möller et al. used GC/MS in positive EI mode for determination of PFRs and plasticizers in North Sea air samples [10]. Separation was achieved using a DB-5MS equivalent column (1 μL injection in pulsed splitless mode on a 30 m column length × 0.25 mm i.d. × 0.25 μm film thickness) with helium as a carrier gas at 1.3 mL min^{-1}. The method LODs (mean blank values + 3 standard deviations) ranged from 1 to 94 pg m^{-3}; however, for some PFRs, the MDLs were one to two orders of magnitude greater for the gaseous phase, compared to the particulate phase, indicating that tandem MS techniques are more suitable for measurements in the gas phase. Internal standard recoveries were 131 ± 46% and 176 ± 40% for filters and 241 ± 130% and 224 ± 15% in air columns for the internal standards $[D_{27}]$-TNBP and $[D_{15}]$-TPHP, respectively. Relative recoveries of PFRs based on correction for the internal standard recoveries ranged from 77 ± 5% for TCEP to 111 ± 4% for TEHP. Möller et al. used the same methodology for determination of PFRs and plasticizers in air particulate from the Northern Pacific and Indian Oceans [6]. Castro-Jiménez et al. used GC/MS in positive EI mode (70 eV) using SIM for determination of PFRs in Mediterranean and Black Sea air. Separation was achieved on a DB-5MS column (2 μL injection on a 30 m column length × 0.25 mm i.d. × 0.25 μm film thickness) with helium as the carrier gas at a flow rate of 1 mL min^{-1}.

4.2.3 Solid Matrices Including Sludge, Dust and Sediment

In their review of PFRs, van der Veen and de Boer provided an overview of five studies that reported the determination of PFRs in sediment [2]. Only one study employed LC/MS, that being the method of Martínez-Carballo et al. who used the same analytical conditions for the determination of PFRs in water; recoveries for sediment ranged from 74% to 104% [48]. Three additional studies were based on GC/MS, including one that used ICP/MS; the fifth study employed GC/NPD. Typical GC separation parameters involved the use of DB-5 equivalent columns (15–30 m length × 0.22–0.32 mm i.d. × 0.25 μm stationary phase thickness) with splitless injection (1–2 μL injection volume). For water, the range of LOQs for the LC/MS (0.48–11 ng g^{-1}) and GC/MS methods (0.10–2,000 ng g^{-1}) compared favourably.

Ballesteros-Gómez et al. have developed high-resolution (HR) TOF/MS methods for solid matrices [64, 87]. These methods were based on GC, two-dimensional GC (2D or GC × GC) and LC. In addition, the authors compared ESI, APCI and atmospheric pressure photoionization (APPI) ionization techniques

in terms of sensitivity and susceptibility to MEs. In the first study, GC/APCI/HRTOF/MS was found to be more sensitive for a suite of FRs, including PFRs, than LC/APCI/HRTOF/MS or LC/APPI/HRTOF/MS; the GC-based method was also reported to be less susceptible to MEs [87]. The 2D-GC separation was advocated for determination of untargeted compounds, i.e. for screening studies. The soft ionization nature of the APCI source resulted in production of $[M+H]^+$ molecular ions in positive mode for most PFRs, with the exception of EHDP ($[M-C_8H_{17}+H_2]$); however, secondary ions were not produced in sufficient quantity for confirmation. Negative ion APCI did not provide adequate sensitivity for chlorinated PFRs. Using the developed GC/APCI/HRTOF/MS method, TCEP was detected at low μg g^{-1} levels in e-waste, while TCIPP was detected at very high levels (200–1,640 μg g^{-1}) in car interiors. In the second study, an LC/APCI/HRTOF/MS method was developed for two specific PFRs, resorcinol bis-(diphenylphosphate) (PBDPP) and bisphenol A bis(diphenylphosphate) (BDA-BDPP), to determine their presence in plastic consumer products [64]. The LC separation was performed on a C18 column (10 cm length × 2.1 mm i.d. × 2.6 μm particle size) using a water/methanol mobile phase (both solvents modified with 0.25% (v/v) formic acid) at 0.3 mL min^{-1}. Values of less than 5 ppm mass error were considered acceptable. For the two PFRs, positive APCI was determined to be between 1.5-fold and 1.7-fold more sensitive than for positive ESI. A suite of other PFRs were also determined using the developed method for comparison to levels of the two compounds of interest. The authors also observed significantly better peak shapes when using a core–shell-based stationary phase as opposed to an equivalent fully porous C18 phase. As with the previous study, both analytes exhibited a predominant $[M+H]^+$ ion in positive APCI. Instrument LODs ranged from 1 to 2 ng mL^{-1} and MEs from 75 to 120%; recoveries were 90%. The PBDPP compound exhibited some ion suppression (75–95% ME), while the BPA-BDPP compound appeared to exhibit enhancement (99–120% ME).

Brandsma et al. compared analysis by GC/EI/MS and LC/ESI/MS/MS in their study of PFRs in car and house dust [64]. The GC separation method used a DB-5 equivalent column (BPX5 25 m length × 0.22 mm i.d. × 0.25 μm film thickness) using pulsed splitless injection and helium at a flow rate of 1.3 mL min^{-1}. The MS was operated in positive EI mode using SIM. The LC method was similar to that described above for PBDPP and BPA-BDPP. The MS was run in MRM mode; the authors presented SIM ions for the GC/MS method and MRM transitions for the LC/MS/MS method. The authors observed good recoveries (79 to 101%) for most PFRs using the LC/MS/MS method, but also >50% signal suppression for four compounds. Recoveries for the GC/MS method ranged from 65 to 112%. The authors recommended the GC/MS method over the LC/MS/MS method. More recently the same methodology was used for determination of PFRs and BFRs and plasticizers in an estuarine food web [77].

Van den Eede et al. developed a GC/EI/MS technique as part of their comprehensive method for determination of BFRs and OPFRs in indoor dust [63]. The PFRs eluting in the second fraction from the cleanup as described previously in the chapter were separated on a HT-8 column (25 m length × 0.22 mm i.d. × 0.25 μm

film thickness) after cold splitless injection; helium was the carrier gas at a flow rate of 1.0 mL min^{-1}. The MS was operated in positive EI mode using SIM; the authors presented both the quantifier and qualifier ions in tabular form. The method LOQs ranged from 10 ng g^{-1} for three PFRs to 370 ng g^{-1} for TIBP; percentages of indicative or certified values for PFRs in SRM 2585 ranged from 97 to 157%. Losses of the volatile TEP and TNBP compounds were observed as a result of the evaporation step for the second fraction. High recoveries for TCEP and TBOEP were attributed to MEs, although these same MEs were only observed in the low-spike level samples; recoveries in the high-spike samples were all acceptable (range of 84 to 110%). The authors also commented on the high LOD and percent RSD (both intra- and inter-day) being typical for TIBP as corroborated in a number of other studies. The method LODs for PFRs were judged by the authors to be similar to those of other older reported methods.

Cristale et al. used GC/EI/MS/MS for the analysis of PFRs and BFRs in sediment, sludge and dust. The GC method was based on a DB-5MS column (2 μL injection on a 15 m column length × 0.25 mm i.d. × 0.10 μm film thickness) with helium as a carrier gas at 1.5 mL min^{-1} [46]. For the tandem MS method, collision energies, precursor and product ions were optimized. As with other studies, the *m*/*z* 99 $[H_4PO_4]^+$ ion was the base peak for many of the PFRs. The authors concluded that the low *m*/*z* ration of the 99 ion was overcome by exploiting the selectivity of the optimized MS/MS conditions in SRM mode vs. SIM mode that is commonly used in GC/MS techniques. Method LODs ranged from 0.0005 to 0.01 ng μL^{-1}. Instrument LODs ranged from 0.4 to 20 pg injected. In terms of instrumental analysis, Cristale et al. used the same separation and detection techniques for the determination of PFRs in water and sediment [88].

Stapleton et al. used previously developed methodology for PFRs in furniture foam and house dust to measure PFRs in furniture as part of a study to assess trends resulting from the 2005 phase out of the penta-BDE formulation [89]. Separation of PFRs by GC was achieved using DB-5 column (PTV injection on a 15 m column length × 0.25 mm i.d. × 0.25 μm film thickness). Analyses were performed in full-scan mode using either GC/EI/MS or GC/ECNI/MS. In the earlier study, TCIPP and TPP were determined using GC/EI/MS, while TDCPP and the BFRs were measured using GC/ECNI/MS. In the later study, TCEP and TDCPP were measured using GC/EI/MS by monitoring the *m*/*z* 249/251 and *m*/*z* 381/383 ions, respectively. The later study also included a LC tandem MS method for the determination of V6, a chlorinated PFR that can contain TCEP as an impurity in the technical mixture. The LC separation was conducted using a C18 column (5 cm column length × 4.6 mm i.d. × 1.8 μm particle size) and a water/methanol mobile phase. V6 was determined using positive APCI in MRM made by monitoring the *m*/*z* 582.7 to 63.0 transition (quantifier) and the 582.7 to 360.8 and 582.7 to 234.8 transitions as qualifiers.

4.2.4 Biological Matrices

The review of van der Veen and de Boer provides five examples of analysis of PFRs in biota; an overview of extraction techniques, extraction solvents, separation conditions, injection modes, detection, LODs (both MDL and IDLs) and recoveries is presented in tabular form [2]. Techniques for analysis included GC/NPD (1 study), GC/MS (3 studies), GC/HRMS (1 study) and LC/MS/MS (1 study). Typical GC parameters included a DB-5 ms or equivalent column (30 m length × 0.25 mm i.d. × 0.25 μm stationary phase thickness) with splitless injection and 1 μL injection volume. Method LODs for the GC/MS-based methods ranged from sub-ng g^{-1} to 40 ng g^{-1}. The method of Leonards et al. based on RP-HPLC (15 cm 3 μm C_{18} column) and LC/MS/MS exhibited the lowest LOQs (0.04–2 ng g^{-1}) [58].

Kim et al. measured PFRs in fish from Manila Bay (Philippines) using UHPLC and LC/MS/MS in positive ESI mode [90]. A 10 μL injection was performed on a C18 column (10 cm column length × 2.1 mm i.d. × 2.7 μm particle size) in conjunction with a 0.1% (v/v) formic acid in water and 10 mM ammonium acetate in methanol mobile phase at 0.2 mL min^{-1}. Recoveries from spiked samples ranged from 58% for tripentyl phosphate to 114% for TEHP; method LODs ranged from 0.001 ng g^{-1} for TPP to 0.014 ng g^{-1} for TPHP.

In their review of analytical methods for analysis of emerging contaminants in human matrices, Dirtu et al. provided an overview of GC/MS parameters for PFR triesters and their metabolites; of the seven studies covered in the overview, the most common combination of parameters was a DB-5 column (5% phenyl – 95% dimethylpolysiloxane, dimensions of 30 m length × 0.25 mm i.d. × 0.25 μm stationary phase thickness) using splitless injection and electron impact ionization. Two studies used a mid-polar DB-35 (35% phenyl – 65% dimethylpolysiloxane) stationary phase, one of which employed derivatization using pentafluorobenzyl bromide (PFB-Br). One other study used derivatization with *N*-methyl-*N*-tert-butyldimethylsilyltrifluoroacetamide (MTBSTFA); derivatization in the analysis of PFRs is used to increase volatility and sensitivity [67]. The study by Sundkvist et al. employed inductively coupled plasma (ICP) for ionization and monitored *m*/*z* 31, which as pointed out by Dirtu et al. was too low a mass to charge ratio to provide structural information, in addition to higher signal-to-noise (S/N) ratios that can negatively impact MDLs [67]. The studies of Schindler et al. employed a GC tandem MS (MS/MS) technique after derivatization with PFB-Br that detected six PFR diester metabolites in plasma with limits of detection (LODs) of 0.1–1.0 ng mL^{-1} [78, 79].

With respect to separation of PFRs and their metabolites using LC, column selection is largely based on the range of polarities of the analytes. Reversed-phase C18 and phenyl-based columns offer good separation for a wide range of compounds; however, the most polar metabolites (BCEP and BCIPP, the dialkyl metabolites of TCEP and TCIPP, respectively) can require a more polar stationary phase, e.g. an ether-linked phenyl phase, for effective chromatography [67]. The seven studies reviewed by Dirtu et al. employed 5–25 cm LC columns with particle

sizes ranging from 2.1 to 5 μm at sub-mL min^{-1} flow rates [67]. Typical mobile phases included water and/or methanol modified with formic acid, tri-*n*-butylamine and acetic acid, and ammonium acetate. Five studies employed electrospray ionization (ESI); four in negative mode. The other studies employed atmospheric pressure chemical ionization (APCI) in positive or negative mode. The APCI ionization techniques exhibited greater reproducibility as they provided similar response factors for PFRs regardless of matrix [67]. The majority of the methods employed tandem MS for detection, with two employing ion trap MS. Detection limits for the tandem MS studies were typically sub-ng mL^{-1}, which represented values roughly an order of magnitude lower than for single MS; the methods based on MS/MS were more contemporary than those based on single MS.

Van den Eede et al. compared GC/MS/MS (positive EI) and LC/MS/MS (negative ESI) for analysis of PFR diester metabolites in human urine [71]. The GC/MS/MS technique was based on that developed by Schindler et al. [78, 79]. Both techniques showed better results for some individual compounds; LC/MS/MS had lower LOQs for DPHP and BBOEP (0.3 and 0.15 ng mL^{-1}, respectively), while GC/MS/MS had greater than an order of magnitude lower LOQs than LC/MS/MS for BCEP, BCIPP and BDCIPP (0.1, 0.06 and 0.02 ng mL^{-1}, respectively, for GC/MS/MS vs. 1.2, 3.7 and 0.5 ng mL^{-1}, respectively, for LC/MS/MS). The authors recommended the LC/MS/MS technique as a general method based on total analysis time; however, GC/MS/MS was recommended for the chlorinated dialkyl phosphates (DAPs) as their hydrophobicity and ionization potentials made them more amenable to this technique. As discussed previously in the chapter, the extracts were derivatized prior to analysis in order to increase retention and sensitivity for LC/MS/MS. The LC separation was based on a ether-linked phenyl phase (polar and aromatic reversed phase, 10 cm column length × 2.1 mm i.d. × 2.0 μm particle size) using a water/methanol mobile phase modified with 10 mM ammonium acetate at a flow rate of 150 μL min^{-1}. The triple quadrupole was operated in negative ESI mode and MRM optimized for each analyte. A range of MEs was observed ranging from 60% signal suppression for BDCIPP to 166% signal enhancement for DPHP. The MEs were able to be accounted for with those analytes for which labelled surrogates were available.

In their review, Dirtu et al. also referred to the method of Chu et al. based on dicationic ion-pairing of PFR diesters post-LC column followed by LC/MS/MS using ESI [67, 79]. This report is the first in an impressive body of methods development work for PFRs and their metabolites in biological matrices conducted collaboratively between Environment Canada and Carleton University. The method was developed to overcome problems encountered during direct analysis by LC/MS/MS in negative ESI mode as previously developed by Quintana et al. in which the dominant fragment anions exhibited *m*/*z* ratios less than 100 and therefore susceptible to interferences. The analytes were separated on a C18 column (10 μL injection on 5 cm length × 2 mm i.d. × 3 μm particle size) using a water/methanol mobile phase; each solvent was modified with 2 mM ammonium acetate [92]. The dicationic reagent solution (0.1 mM aqueous solution of decamethonium hydroxide) was introduced using a syringe pump (5 μL min^{-1} flow rate) and a

T-connector post-column in order to form ion pairs. Detection was achieved using quadrupole-time-of-flight (Q-TOF) mass spectrometry in positive ESI mode using MRM. The method was developed for four PFR diesters including DPHP, DBP, DDCP and DEHP, and the authors identified the most abundant ions for the phosphate acid diester ion pairs and some of the fragment ions. The LOQs for the method ranged from 0.02 to 0.14 ng mL^{-1}. The authors expressed concern that production of the desired dicationic ions could be impeded by competition from other cations in the matrix, i.e. matrix effects; however, based on experiments of in vitro metabolism, it was determined that matrix effects were negligible. In a continuance of their work on development of methods for "lipid-rich" samples, Chen et al. used LC/MS/MS in positive ESI mode for the determination of a suite of non-halogenated, chlorinated and brominated PFRs in herring gull eggs; a key component of the method was the two-stage extraction and cleanup method discussed previously that afforded extracts amenable to achieving maximum analytical performance [77]. The method used phenyl column (10 μL injection on a 10 cm length × 2.1 mm i.d. × 3.5 μm particle size) and a gradient elution programme using a water/methanol mobile phase at 0.2 mL min^{-1}; both solvents were modified with 0.1% formic acid (v/v). Detection was performed in SRM mode based on the most abundant precursor/product ions for each compound. Matrix effects were measured using a standard addition approach with chicken egg homogenates; mean signal recoveries ranged from 89% to 106% for most compounds indicating minimal matrix effects. The recoveries ranged from 89 to 104%, with the exception of lower recoveries for the brominated compounds (67 to 72%), and method LOQs ranged from 0.06 (wet wt.) to 0.20 ng g^{-1} (wet wt.). The method enabled determination of PFRs using only 1 g sample sizes.

Su et al. used a combination of three LC/MS techniques to study the in vitro (chicken embryonic hepatocytes) metabolism of TPHP; the parent and diphenyl hydrolysis metabolite (DPHP) were determined by LC/MS/MS in positive ESI mode, the hydroxylation metabolites by LC/Q-TOF in positive ESI mode and phenyl phosphate (PHP) and the hydroxylation metabolites by UPLC/MS/MS in positive ESI mode [81]. Quantification of TPHP and DPHP by LC/MS/MS, including the dicationic method for DPHP, was achieved using LC/MS/MS methods by the same group, as previously discussed [81, 91]. Determination of the hydroxylation metabolites (OH-TPHP and $(OH)_2$-TPHP) was by LC/Q-TOF using a C18 column (10 μL injection volume on a 5 cm length × 2 mm i.d. × 3 μm particle size) and a water/methanol mobile phase at 0.4 mL min^{-1}; each solvent was modified with 2 mM ammonium acetate. Mass resolution for the Q-TOF was >20,000at m/z 322.0481 using the described MS conditions. The UPLC separation for PHP and the hydroxylation metabolites (OH-TPHP and $(OH)_2$-TPHP) used a C18 column (5 cm length × 2.1 mm i.d. × 1.6 μm particle size) at a flow rate of 0.7 mL min^{-1} using the same mobile phase as for the Q-TOF method. The authors were unable to accurately quantify the hydroxylation metabolites due to the lack of authentic standards for calibration. In a follow-up study, Su et al. used the UPLC/MS/MS for the determination of MeO-TPHP isomers in addition to the OH-TPHP isomers and conjugates [79]. Separation conditions were similar to those of the previous study with

detection in positive ESI mode using MRM. In this most recent study, the authors were able to perform an external calibration using *p*-OH-TPHP and *m*-OH-TPHP; method LOQs based on S/N ratios of 3 and 10 for both isomers were 0.03 and 0.01 ng mL^{-1}, respectively. The most recent work by this group for the development of dispersive SPE for biological samples as previously discussed also compared ESI and APCI ionization for PFRs [41]. Matrix effects (MEs) for a UPLC/MS/MS method in positive APCI ranged from 40 to 94%, while MEs ranged from 0 to 36% for positive ESI. For the APCI method, method LOQs ranged from 0.06 to 0.29 ng g^{-1} in egg samples to 0.0.5 to 0.50 ng g^{-1} in liver samples. The study indicated that APCI could be far less susceptible to MEs than ESI in the determination of PFRs.

To overcome the issue of MEs when using ESI, Reemtsma et al. performed standard addition in using the method of Quintana et al. for the determination of 14 PFR compounds, including monoalkyl phosphates (MAPs), DAPs and dialkylthiophosphates, in human urine [92, 93]. The authors identified precision, rather than S/N ratio, as the limiting factor for sensitivity in LC/MS. Median LOQs for human urine ranged from 0.3 to 11 ng mL^{-1} in urine. Van den Eede et al. used ultrafast LC (UFLC) with a C8 column (2 μL injection on a 10 cm column length × 2.1 mm i.d. × 1.7 μm particle size) in conjunction with an acetonitrile/water mobile phase modified with 5 mM ammonium acetate at a flow rate of 0.25 mL min^{-1} and a temperature of 55°C for the determination of PFR metabolites in human urine [71, 73]. The triple-quadrupole MS was operated in positive ESI mode. The MDLs were generally <0.5 ng mL^{-1}; however, values exceeded 3 ng mL^{-1} for BCEP and BCIPP which precluded reporting of concentrations for these compounds. With the exception of these two compounds, overall recoveries and precision were judged to be satisfactory for the remaining analytes.

Kim et al. applied UHPLC/MS/MS methodology to analysis of PFRs in fish and human breast milk [76, 90]. Separation was achieved on a C18 column (10 μL injection volume on a 10 cm column length × 2.1 mm i.d. × 2.7 μm particle size) using a 0.1% formic acid (v/v) in water and 10 mM ammonium acetate in methanol mobile phase. For the fish study, two analytical runs were performed, one with the MS in positive ESI mode and the other in negative ESI mode [90]. The LC mobile phase flow rate was lower for the positive ion run (0.2 mL min^{-1}) than for the negative ion run (0.3 mL min^{-1}). The authors presented substantial QA/QC information, as well as information on the experimental parameters including MRM transitions, declustering potentials and collision energies. The QA/QC data included linearity, precision, recovery, inter- and intra-day RSDs, MDLs at both 3× blank and 10× blank SDs and MEs. The 3× blank MDLs ranged from 0.2 to 15 pg g^{-1} and MEs ranged from 76.8 to 121%. For the human breast milk study, recoveries and RSDs of native compounds at 1 ng ranged from 70 to 112% and 3.1 to 14%, respectively, while MDLs ranged from 0.01 ng g^{-1} lipid to 0.08 ng g^{-1} lipid [76].

4.3 Blank Contamination

The potential for blank contamination is a critical consideration in methods development for PFRs. The blank contamination issue appears to be acknowledged by the global environmental analytical community at large, but many laboratories suffer these impacts to widely varying degrees. Brandsma et al. published the results of the first worldwide interlaboratory study for PFRs and observed that high blank values in some individual laboratory results contributed to higher coefficients of variation (CVs), rather than the CVs being correlated with any specific matrix type [38]. However, they also observed that some laboratories did not suffer from blank contamination which led them to determine that mitigation of blank issues is possible.

Brandsma et al. determined the issue of blank contamination to be important enough to include a separate discussion, including mitigation measures, in their report [38]. In general, TBP, TiBP, TBEP and total-TCIPP were the most predominant compounds in the blanks reported for the interlaboratory study. It is now commonplace to report blank values, particularly for those compounds that are particularly problematic, in studies describing the occurrence, distribution and fate of PFRs. For example, Cristale et al. reported TCIPP and TPHP in procedural blanks at levels of 15.5 ± 1.3 and 3.5 ± 0.3 ng L^{-1}, respectively, and performed blank correction on data for surface water [88]. High blank levels of TBP encountered by Brandsma et al. in their study of BFRs, plasticizers and PFRs in an estuarine food web precluded reporting data for this compound [78]. Möller et al. determined absolute blank levels of TCIPP of $5{,}200 \pm 140$ pg in their study of PFRs and plasticizers in air from the northern Pacific and Indian Oceans, while Castro-Jiménez et al. found that TPHP background contamination reached approximately 40% of actual sample values in their study of PFRs and plasticizers in air from the Mediterranean and Black Seas. Su et al. even found "unavoidable" background contamination for PFR diesters in their study of PFR metabolites in biotic samples [6, 52, 80].

The varying degrees of blank contamination observed globally in analytical laboratories indicate a myriad of sources, and environments can contribute to blank contamination in the analysis of PFRs. Optimally, sample preparation for analysis of PFRs should be conducted in a cleanroom laboratory environment, or ultra-clean cabinets with positive pressure, if available. Brandsma et al. and other analysts have made recommendations to reduce the degree of background contamination [54]. Since indoor dust can contain significant burdens of PFRs, minimizing exposure of samples, laboratory glassware and equipment to dust is an important consideration. TBEP is the most prevalent PFR in household dust and is also one of the compounds most typically problematic in blanks [38]. Indoor dust PFR concentrations can also be highly variable, even in different rooms in the same building, which could be a factor in the wide range of PFRs that can be problematic in terms of background contamination; this observation also indicates the varying

influences of indoor sources including furniture, insulation and electronics [38]. Cao et al. determined these variations can also be seasonal in nature [59].

Brandsma et al. recommended precleaning of glassware, glass frits in Soxhlet extraction thimbles and metal frits in ASE cells with both polar and non-polar solvents including hexane, acetone and methanol [38]. They also recommended precleaning of silica, Florisil, aminopropyl silica or alumina columns and/or SPE cartridges with dichloromethane, methanol, ethyl acetate or a mixture thereof. Similarly, van der Veen and de Boer recommended ultrasonication of GFFs in methanol, dichloromethane or acetone [2]. However, the fact that solvents can be contaminated with PFRs must also be taken into account as they themselves represent a potential source of contamination. Glassware reportedly can be cleaned using ethanol with 5% (w/w) sodium hydroxide or 5% (w/v) of a nonionic surfactant followed by rinsing with water, ethanol and acetone [2, 44, 51]. For air sampling, PUFs can be rinsed with water, acetone and dichloromethane subsequent to Soxhlet extraction in dichloromethane [2, 51, 94]. It is now commonplace in large-scale monitoring programmes to have air sampling media cleaned and prepared by third-party contractors in cleanroom environments, sealed and shipped to the laboratory for deployment in the field. Another factor impacting method performance for PFRs is the tendency for some compounds to adhere to glass, e.g. TBP which indicates use of glass in preparation of samples for some PFRs should be avoided [2, 51]. Teflon should also be avoided [2].

Exposure of samples and/or extracts to plastics and rubbers, including tubing, septa and O-rings, can also be sources of PFRs [2, 54]. In the development of a method for determination of BFRs and PFRs in sediment, sludge and dust, Cristale et al. baked centrifuge tubes, vials and pipettes overnight at 350°C to minimize blank contamination. Plastic materials were avoided and samples and glassware were covered with aluminium foil to avoid exposure to dust [46]. Möller et al. also avoided usage of any plastic and rubber materials and used air sampling equipment made exclusively of stainless steel [6]. Air filters were baked at 450°C for 12 h and glassware was baked at 250°C for 12 h and subsequently rinsed with acetone. Silica gel was cleaned with dichloromethane for 12 h and then baked at 450°C for 12 h.

Dirtu et al. also provided recommendations regarding precautions to be taken in minimizing blank contamination in the analysis of PFRs and metabolites in human matrices. Materials used in sample collection must be considered as PFRs can leach from PVC bags or be adsorbed to plastic containers including those made of polypropylene [67]. DNBP and DPP were both identified as being used as plasticizers in some polymers. While van der Veen and de Boer recommended against use of Teflon in PFR methods, Dirtu et al. recommended its use for caps, filters and frits [2, 78]. They also recommended use of glass columns for SPE as opposed to plastic cartridges; if plastic SPE cartridges are used, they should be rinsed with a highly polar solvent prior to use. In sum, all laboratory materials should be checked for blank contamination prior to their incorporation into any methodology for PFRs.

4.4 General Considerations for the Analysis of PFRs

The wide range of physical and chemical properties of PFRs makes it difficult to apply a single method for a comprehensive approach to analysis. In addition, the recent interest in determination of PFR metabolites in biological matrices has widened the range of properties to be considered in experimental design. As with other compound classes that have found heavy use in industrial and consumer products, the ubiquity of PFRs can result in significant background contamination in modern laboratory environments that in turn has led to a trend toward designing extraction and cleanup steps that minimize extraction and/or cleanup solvent volumes. van der Veen and de Boer recommended SPE for extraction and/or cleanup based on generally satisfactory recoveries and RSDs, reduced solvent volumes compared to LLE and the potential for using automated laboratory systems that can also be coupled to analytical instrumentation [2]. The use of large volume injection (LVI) may provide an alternative to SPE methods as the comparison of MEs using the two techniques showed that analytical signals were similar; LVI was promoted as being lower cost, less labour-intensive and eliminated analyte loss associated with SPE [95]. However, PFRs were not included in the analyte suite in the study.

Ma and Hites undertook a systematic assessment of EI, electron capture negative ionization (NICI) and positive chemical ionization (PCI) of thirteen PFRs in order to investigate fragmentation and ion structures as had been done previously for PBDEs [96]. A thorough understanding of mass spectral fragmentation mechanisms leads to development of more efficient and sensitive GC/MS methods. For the selected compounds, EI spectra were dominated by $H_4PO_4^+$, $[M\text{-}Cl]^+$, $[M\text{-}CH_2Cl]^+$ or $[M]^+$; the relative abundances of these ions were dependent on chemical structure. The ECNI spectra were generally dominated by $[M\text{-}R]^-$ ions, while PCI spectra were generally dominated by the $[M+H]^+$ ions. Specific aspects of chemical structure including branching of alkyl substituents, halogenation of substituents and *ortho*-alkylation of ring structures for aromatic phosphate esters were determining factors in fragmentation. Overall, the authors determined that EI exhibited the greatest sensitivity; however, *m*/*z* 99 was a prevalent fragment ion that provided less value in terms of structural identification. van der Veen and de Boer and Brandsma et al. also identified extensive fragmentation for alkylated phosphates as a detriment to structural identification [2, 47]. The *m*/*z* 99 fragment ion is the result of three successive McLafferty rearrangements of alkyl phosphate ester ion–dipole complexes [38, 96]. The low *m*/*z* ratio of this fragment ion also makes it susceptible to inferences in complex matrices.

Ma and Hites determined that halogenated alkyl PFRs tended to fragment thru loss of Cl or Br. In some cases, ECNI and PCI exhibited good selectivity, but with poor relative sensitivity [96]. The presence of $[M\text{-}R]^-$ and $[M\text{-}H]^-$ ions when using ECNI, and the presence of the $[M+H]^+$, $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$ ions when using PCI contributed structural information. The issue of greater selectivity, but poorer sensitivity, for PCI methods had been previously identified by a number of

groups [2, 84, 94]. However, even in SIM mode GC/MS in EI mode can be susceptible to interferences [97]. The use of GC/MS/MS and/or GC/HRTOF/MS methods and the use of MRM would certainly alleviate some of these issues related to interferences. Ultimately the selection of ionization technique will be determined by data quality objectives and the balance between the requirement for high sensitivity and good selectivity; all of which will be matrix dependent.

Of the 15 participating laboratories in the first worldwide interlaboratory study for PFRs and plasticizers, 9 laboratories used GC/MS in positive EI mode and 6 laboratories used LC/MS/MS [38]. Using ANOVA ($p > 0.05$) there were no significant differences for all matrices in mean concentrations between the GC/MS and LC/MS/MS techniques, with the exception of total TCP in dust. Brandsma et al. also discussed the issue of sensitivity vs. selectivity for GC/MS in EI mode and PCI mode [38]. Quantification of PFRs such as TBP, TIBP and TEHP using GCMS in positive EI mode must be performed with less sensitive fragment ions. They also referred to work by Bergh et al. who observed decreased response times for TCEP, TDCPP and TBOEP due to degradation on the GC column [98]. In addition, short-chain alkyl PFRs (TIBP, TBP) exhibited tailing over time which emphasized the importance of the cleanup method and the requirement for frequent inter-run instrument performance checks.

Brandsma et al. also addressed the issue of MEs when using LC/MS analysis and identified this issue as a disadvantage; however, they also indicated that MEs are less important when labelled surrogates are available to correct for ion suppression or enhancement [38]. The number of commercially available labelled surrogates for PFRs continues to increase which in turn will enable better compensation for MEs and more accurate and precise data for PFRs while exploiting the substantial resolving power, sensitivity and selectivity of LC/MS/MS.

As with some other novel compound classes, LC/MS analysis of PFRs can be susceptible to formation of stable complexes with metal cations including Na^+ that can result in formation of $[M+Na]^+$ and $[2\,M+Na]^+$ ions [2]. Formation of these ions is dependent on a number of factors including pH and availability of metal cations in the sample and/or mobile phase [99]. The presence of these complexes can reduce sensitivity and must be monitored to mitigate their impact on overall method performance. Analysts have investigated the potential for using these complexes as a means of quantification as these ions can be predominant in some cases; however, formation of these complexes can also be highly variable which makes them unsuitable for qualitative determination.

As with selection of extraction and cleanup methods, ultimately the choice of instrumental analysis technique(s) will be dependent on the range of physical and chemical properties of the entire suite of analytes, in addition to the required data quality objectives. Techniques based on GC/EI/MS (with or without derivatization), GC/ECNI/MS, GC/PCI/MS, LC/MS/MS using ESI or LC/MS/MS with APCI may be entirely appropriate for instrumental analysis.

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Index

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