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Detecting organic contaminants in food: the case of fish and shellfish

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21.1 Introduction: the problem of organic contaminants

Over the last two decades, organic contaminants in the environment and food have gained much attention from scientists, policy makers, manufacturers and environmental non-governmental organisations. From a food safety point of view, crises on contaminants like the Belgian dioxin crisis (1999), in which polychlorinated biphenyls (PCBs) entered the food chain as a result of using PCB-contaminated oil for production of feed, have increased consumers' concern on contaminants in their food. Fish and shellfish are susceptible to contamination with undesired substances that have entered the aquatic environment prior to, during or after use and production of these substances.

The importance of contaminants with regard to the health of humans is normally based on the following criteria: persistence; bioaccumulation and toxic effect. Another aspect that should be taken into account is the production volume of the chemicals. A very toxic substance may be of relatively low importance when its production volume is very limited. The production and use of some of the contaminants discussed in this book, such as PCBs, was stopped years ago, whereas substances with comparable properties and behaviour are still produced and used in high quantities, like polybrominated diphenyl ethers (PBDEs).

This chapter will discuss concentrations of a wide range of organic contaminants in fish and shellfish. Furthermore, the sources and contamination of fish will be discussed as well as general principles of analysis. Finally, future trends and sources for further information are briefly addressed.

21.2 Sources of organic contaminants

Organic contaminants originate from a variety of sources. With the exception of halogenated dibenzo-p-dioxins (PCDDs) and – furans (PCDFs) and polycyclic aromatic hydrocarbons (PAHs), most organic contaminants have originally been manufactured as formulations for specific applications. In most cases these formulations are complex mixtures with varying degrees of halogenation, depending on the physicochemical properties required by the application.

Most manufactured contaminants are made from the chlorination (or bromination) of the parent hydrocarbon in the presence of an appropriate catalyst. The production, properties and usage of each group of contaminants have been extensively reviewed and is well documented (Brinkman and de Kok, 1980; WHO, 1992, 1994a, b; Erickson, 1997). The primary sources of these compounds are in many cases interdependent, and examples are now discussed.

21.2.1 Direct manufacture for use in either open or closed systems

Various compounds and formulations have been manufactured for use in open systems such as additives in plastics or sealed systems as transformers. This group comprises PCBs, polychlorinated terphenyls (PCTs), polybrominated biphenyls (PBBs), PBDEs, polychlorinated alkanes (PCAs) and polychlorinated naphthalenes (PCNs). These contaminants span a wide application range including use as plasticisers, flame retardants, lubricants, in cutting oils in metal works, dielectric fluids, paper coatings and printing inks. For example, PBDEs are generally applied as flame retardants in materials to prevent them from catching fire (de Boer, 2000a, b, c), whereas PCAs find their application as, for example, plasticiser in polyvinyl chloride (PVC), rubber paintings and as extreme pressure lubricant in the metal working industry (Slooff *et al.*, 1992; Muir *et al.*, 2000; Poremski *et al.*, 2001).

Musk compounds find their application as synthetic fragrances in personal care products and alkyl phenolic compounds (and their ethoxylates) originate from application as, for example, detergents (Staples *et al.*, 1998).

21.2.2 Impurities in manufacture and subsequent use of the primary product

PCDDs are present as impurities in pentachlorophenol (PCP) used as a wood preservative, and in herbicides such as Agent Orange which is used as a defoliant. PCNs and polybrominated naphthalenes (PBNs) are present as impurities in PCB and PBB manufacture, respectively. In addition, PCDDs and PCDFs are present as impurities in the high temperature chlorination manufacture of other chlorinated contaminants. Unlike the PBDEs, the chlorinated homologues (PCDEs) are not manufactured directly for commercial use, but they are an impurity of technical chlorophenols primarily produced as wood preservatives.

21.2.3 Formation through the disposal of other contaminants

PCDDs and PCDFs are formed in the incineration of material containing PCBs, PCNs and PVC. PCDDs and PCDFs are not manufactured and have no commercial use. They are formed as by-products of incineration of waste products, combustion of sewage sludge and fires containing chlorinated materials such as PCBs, PVC, PCP and PCDEs (Fiedler *et al.*, 1990). Most of these compounds are formed in the incineration process when the temperature is not sufficient to completely destroy the parent compounds. A similar process occurs with brominated flame retardants (BFRs) such as PBBs and PBDEs from which polybrominated dibenzodioxins (PBDDs) and dibenzofurans (PBDFs) are formed (Sakai *et al.* 2001).

21.2.4 Direct manufacture for use as pesticides

Organochlorine pesticides (OCPs) have been produced mainly for agricultural applications. The group of OCPs comprises a variety of compounds like aldrin, dieldrin, lindane, DDT, toxaphene and hexachlorobenzene (HCB).

21.3 Organic contaminants in fish

21.3.1 Accumulation of organic contaminants in fish

The main source of contaminants is via uptake through the aquatic food web to fish and shellfish. Due to the lipophilicity of most of the contaminants, these substances are well absorbed in the lipids of fish. The uptake can take place either directly from the surrounding water or by uptake from food (bioaccumulation) (Geyer *et al.*, 2000), leading to higher concentrations in fish compared with their food or surrounding water. Concentrations can increase further when the fish serves as food for other (bigger) carnivorous species, leading to biomagnification (Geyer *et al.*, 2000) in the food chain.

For farmed fish the main source of contamination is the feed, which can be composed of potentially contaminated ingredients like fish meal and fish oil. Fish oil originating from European waters shows dioxin concentrations about 10 times higher compared with oil originating from the Pacific (SCAN, 2000). In particular, the diet of carnivorous species contains substantial amounts of fish oil and meal, leading up to 98% of relative contribution of fish oil and fish meal to the contamination of fish with dioxins (SCAN, 2000). A recent study showed considerable levels of dioxins, PCBs, toxaphene and OCPs in European farmed salmon which are likely to relate to the elevated levels of these contaminants in European salmon feed (Hites *et al.*, 2004).

The lipophilic contaminants are generally very persistent and fish show limited ability to eliminate these contaminants by diffusion or by transformation (e.g. in the liver followed by excretion). An exception are the PAHs which are metabolised in the gall-bladder and subsequently excreted (Baussant *et al.*, 2001) and are therefore generally not found in fish. However, shellfish have no ability to metabolise PAHs and therefore these substances accumulate in shellfish.

21.3.2 Polycyclic aromatic hydrocarbons (PAHs)

As described above, PAHs do not normally occur in fish and are, therefore, of no importance with respect to human health. Shellfish, however, metabolise PAHs only to a minor extent and therefore accumulate PAHs.

There is no recent data on concentrations of PAHs in Dutch mussels (see Table 21.1), but data from 1991 shows concentrations up to 6.8 μg/kg wet weight (ww) for the sum of 13 PAHs and concentrations below 1 μg/kg ww for the carcinogenic benzo(a)pyrene. PAH concentrations reported from the Mediterranean are extremely high as they are expressed on a dry weight (dw) basis (see Table 21.1). There is only limited data available from scientific publications and therefore, the current situation concerning PAHs in mussels and other shellfish is not clear, which shows the need for new data.

21.3.3 Polychlorinated biphenyls (PCBs)

The group of PCBs is a complex mixture, consisting of 209 congeners. A representative selection of congeners, the seven so-called indicator PCBs (CB 28, 52, 101, 118, 138, 153 and 180) are regularly analysed in fish samples and are often included in monitoring programmes. Therefore, a vast amount of data is available (see Table 21.2).

Eel samples from polluted areas in the Netherlands like Haringvliet, river Meuse, river Rhine and Western Scheldt show high concentrations of PCBs up to 730 μg/kg ww for CB 153, occasionally exceeding Dutch maximum residue limits (MRLs) (Anon., 1984). Fish originating from other places than the contamination hot spots (e.g. industrialised areas and polluted rivers) generally show significantly lower concentrations. Marine fish generally show lower concentrations compared with freshwater fish due to dilution of the more contaminated river effluents (Atuma *et al.*, 1996; Ylitalo *et al.*, 1999; Leonards *et al.*, 2000).

For farmed salmon there was no clear difference in PCB concentrations compared with wild salmon from Scotland. Concentrations of PCBs in farmed salmon were both higher and lower than the investigated wild salmon samples (Jacobs *et al.*, 2001a, b). This was also observed for OCPs and PBDEs in farmed and wild salmon (Jacobs *et al.*, 2001a, b). Clearly, concentrations in farmed salmon depend very much on the origination of fish oil and fish meal used in the feed. Fish oil and meal from the Pacific generally contain lower concentrations of contaminants compared with fish oil from Europe (SCAN, 2000). In another study the concentrations of contaminants (including PCBs) were significantly higher in European salmon compared to salmon from North America (Hites *et al.*, 2004).

Since the production and use of PCBs stopped in the early 1980s, temporal trends show clearly declining PCB concentrations in freshwater and marine fish up to a

Table 21.1 Typical concentrations of PAHs in fish and shellfish ($\mu g/kg$)

Country	Location	Year	Species	BaP*	DahA	BaA	BbF	BkF	IP	Ch	Fluo	P	Py	ww/dw**	References
The Netherlands	Various	1991	Mussels	0.6	< 1	1.2	4.7	1.2	1.1	1.9	6.4		6.8	ww	LAC (1991)
Italy	Mediterranean Sea	1999	Mussels	7–127		5.7–250	12–218	14–98		19–262	67–924	21–436	29–351	dw	Piccardo *et al.* (2001)
France	Mediterranean Sea	1995	Mussels	0.2–9.7	0.24–2.9***	0.11–16	2–39****		0.62–7.5	3.6–142	1.2–20	7.4–86	1.2–53	dw	Baumard *et al.* (1998)

* BaP: benzo(a)pyrene, DahA: dibenzo(a,h)anthracene, BaA: benzo(a)anthracene, BbF: benzo(b)fluoranthene, BkF: benzo(k)fluoranthene, IP: indeno(1,2,3-c,d)pyrene, Ch: chrysene, Fluo: fluoranthene, P: phenanthrene: Py: pyrene

** ww: wet weight and dw: dry weight

*** Including dibenzo[a,c]anthracene

**** Including benzo[j]fluoranthene and benzo[k]fluoranthene

Table 21.2 Typical concentrations of PCBs in fish (μg/kg)

Country	Year	Species	CB 28	CB 52	CB 101	CB 118	CB 138	CB 153	CB 180	Sum	ww/dw	References
Sweden	1992–93	Various	< 0.05–0.38	< 0.05–2.3	0.09–7.9	0.07–6.6	0.25–13	0.35–20	0.08–4.8		ww	Atuma *et al.* (1996)
		Eel	0.81	5.4	19	30	50	69	19			
Barents and Greenland Sea	1991–92	Various	ND–1.8	0.1–8.2	0.5–42	0.3–16	1.3–29	0.2–22	0.7-6.8	6.0–113 (11)*	dw	Ali *et al.* (1997)
Netherlands	1999	Eel	<0.7–12	1.5–110	1.7–160	2.5–160	6.3–390**	9.0–730	2.9–270		ww	de Boer *et al.* (2000a)
Netherlands	2000	Various marine and freshwater	< 0.1–8.7	< 0.1–42	< 0.1–77	< 0.1–77	0.04–130	< 0.1–200	< 0.1–74		ww	Leonards *et al* (2000)
UK, Mersey estuary, Liverpool Bay	1990–92	Various vertebrates	0.2–1.1	0.1–1.9	0.7–3.7		3–4.5	2.7–4.9	1.2–2.2		ww	Leah *et al.* (1997)
West of UK	1991–94	Mackerel			5.0–8.7	ND-6.8	4.8–17	5.7–22	1.3–5.6		ww	Karl and Lehman (1997)
USA, Great Lakes	1993?	Various		1.4–6.9		3.3–8.5	8.9–20	3.5–19	4.0–10***			
USA, San Francisco Bay	1994	Various fish from anglers								17–638****	ww	Fairey *et al.* (1997)
USA, North/South Atlantic and Monterey Bay	1992–98	Various	0.3–9.1	0.9–36	3.1–70	3.2–56	13–176	7.6–234	9.6–90	50–1400 (24)	lw	Froeschesies *et al.* (2000)
USA, Altantic/ Pacific coast	1993–94	Various			0.39–14	0.28–5.5	ND-7.8	0.43–19	0.20–6.0		ww	Ylitalo *et al.* (1999)
North America, Europe	2001–02	Farmed salmon								Up to 55 (n = ?)	ww	Hites *et al.* (2004)

ND: not detected; * In brackets: number of summarised congeners; ** Sum CB 138 and CB 163; *** Sum CB-180 and CB 193; **** Expressed as the sum of Arochlors 1248, 1254, 1260 and 5460 factor of four in North-America and Europe (Picer and Picer, 1994; de Vault *et al.*, 1996; Roose *et al.*, 1998; de Boer *et al.*, 2000a). From Table 21.2 it is clear that CB 138 and CB 153 are the predominant congeners in fish samples.

21.3.4 Polychlorinated dibenzodioxins (PCDDs) and -furans (PCDFs) and dioxin-like PCBs (dl-PCBs)

The availability of data within the European Union (EU) on dioxins has increased considerably after the Belgian dioxin crisis in 1999. Following this crisis, legislation on dioxins in food and feed has become active in the EU since 1 July 2002. The MRLs for fish and fish oil for human consumption are 4 pg PCDD/F-toxic equivalents (TEQ)/g ww and 1.5 pg PCDD/F-TEQ/g lw, respectively. Dioxin-like PCBs (dl-PCBs) are not included in this MRL, but will be included in legislation in the future (2004).

Biological samples often contain a complex mixture of PCDD/Fs and dl-PCBs. 2,3,7,8–TCDD is the most potent dioxin-congener with a the toxic equivalency factor (TEF) of 1 and the potency of other congeners or dl-PCBs is equal or lower. The concentration of individual congeners multiplied by their TEF factors results in the total concentration of dioxins and dl-PCBs, expressed in TEQs (van den Berg *et al.*, 1998). The TEQ principle has been discussed in more detail in Chapter 20.

The data from the SCOOP assessment (see Table 21.3) show that most fish samples were well below the EU MRL, although a crab sample from Sweden and Baltic salmon showed concentrations of 10.2 and 7.0 pg TEQ/g product, respectively (SCOOP, 2000). Data on dl-PCBs was limited probably due to the fact that accurate methods of analysis have only become available in the last 5–10 years (Hess *et al.*, 1995). High concentrations of dioxins and dl-PCBs were also found in pike perch from the Netherlands, farmed salmon and tuna from the Mediterranean (Leonards *et al.*, 2000), herring from Scotland (Parsley *et al.*, 1998) and some samples from Asia (Sakurai *et al.*, 2000; Jeong *et al.*, 2001).

Eel from several polluted main rivers in the Netherlands showed high concentrations of dioxins and dl-PCBs up to 7.9 and 44 pg TEQ/g ww, respectively, whereas in lakes and smaller rivers concentrations were considerably lower (van Leeuwen *et al.*, 2002). Concentrations of dioxins and (dl-)PCBs in farmed eel were within the range of the wild eel samples (van Leeuwen *et al.*, 2002). Concentrations of dioxins and dl-PCBs in farmed eel and imported eel were below the EU MRL. The dl-PCBs in the eel samples contributed significantly more (average *ca.* 80%) to the total TEQ compared with the contribution of dioxins. Also in fish other than eel dl-PCBs generally predominantly contribute to the total TEQ, although contribution figures have a wider range (from *ca.* 50–95%) (Leonards *et al.*, 2000). As far as future EU legislation is concerned, it is not yet clear how future MRLs will evolve when dl-PCBs are included in legislation (expected by the end of 2004).

Table 21.3 Typical concentrations of PCDDs, PCDFs and dioxin-like PCBs in fish and shellfish (ng TEQ/kg)

Country	Year	Species	WHO / I	PCDD/F	PCB	Sum	lw/ww	References
Denmark	1995-99	Various	I	0.01–1.6			ww	SCOOP (2000)*
Finland		Trout, farmed		0.74	1.5			
Norway		Crab		10.2				
Sweden		Various		0.13–7.0**	0.23–9.1**			
UK		Various		0.03–2.1	0.07–6.2			
Italy		Various		0.1–0.86				
Netherlands	2002	Eel, freshwater	WHO	0.21–7.9	0.7–44	0.89–52		van Leeuwen *et al.* (2002)
		Eel, farmed		0.9–3.1	2.8–7.7	3.9–11		
North Sea	2000	Various marine and freshwater fish	WHO	0.01–3.9	0.02–33	0.03–37	ww	Leonards *et al.* (2000)
Scotland	1995–96	Cod, haddock, plaice, whiting	WHO	0.01–0.52	0.01–0.85	0.02–1.3	ww	Parsley *et al.* (1998)
		Salmon		0.57–0.99	1.3–3.0	2.2–3.9		
		Mackerel		0.14–1.7	0.34–6.0	0.48–7.5		
		Herring		0.34–3.8	0.46–10	6.8–14		
Italy, Adriatic Sea	1997–98	Various	I	0.07–1.1			ww	Bayarri *et al.* (2001)
Japan, Tokyo bay	1995	Various fish	I	0.32–2.1			ww	Sakurai *et al.* (2000)
		Japanese cockle		3.6				
		Crab		2.6				
Korea, Main river systems	1999–00	Crucian		ND–4.1			ww	Jeong *et al.* (2001)
		Minnow		ND–1.1				
Korea, market fish	1999	Various		0.013–0.58			ww	Ok *et al.* (2001)
Korea, major cities	2000?	Various salt water and shellfish	WHO	0.001–2.9	0.001–6.7		ww	Choi *et al.* (2001)
North America, Europe	2001–02	Salmon, farmed	WHO			1–3.3	ww	Hites *et al.* (2004)

* National average concentrations of 1995–1999 data

** High concentrations originate from Baltic salmon

21.3.5 Polybrominated diphenyl ethers (PBDEs) and other brominated flame retardants (BFRs)

Although concentrations of PBDEs in fish have been determined since the late 1970s (de Boer and Dao, 1993), considerable data have only recently become available due to an increased awareness of the environmental impact of PBDEs (Luross *et al.*, 2002; Rice *et al.*, 2002; de Boer *et al.*, 2003; Hale *et al.*, 2003). From Table 21.4 it is clear that, generally, concentrations of the tetra- and penta-BDEs are predominant over concentrations of higher brominated BDEs, due to the use of the penta-mixture mainly consisting of tetra and penta-BDEs (de Boer, 2000b).

The concentration of the various BDEs in freshwater and marine fish ranges from the mid ng/kg to mid μg/kg range. Generally, when levels are transformed to a wet weight (or product) basis, it can be concluded that the levels in fish from the Great Lakes (Canada) and the Baltic Sea are higher compared to levels in samples from other locations. Among the Great Lakes, Lake Ontario showed considerably higher levels than the other lakes, which might be explained by possible local point sources in the heavily urbanised lake basin (Luross *et al.*, 2002).

De Boer and Allchin (2001) established temporal trends of PBDEs in eel originating from the Haringvliet-East in the Netherlands and found an approximate eight-fold decrease in concentrations of BDE 47 from 1979 to 1999 (*ca.* 1200 to *ca.* 150 μg/kg lw). On the other hand, exponential increases of tetra-, penta- and hexa-BDE concentrations were detected between 1981 and 2000 in ringed seals in the Canadian Arctic (Ikonomou *et al.*, 2002), reflecting the continuing production and use of high volumes of the technical penta-BDE mixture in North America (Alcock *et al.*, 2003; Hale *et al.*, 2003).

Jacobs *et al.*, (2001a) determined 9 BDE congeners in 13 samples of farmed and wild salmon (Norway, Scotland, Ireland and Belgian market) and found concentrations ranging from 5.0 to 43 μg/kg ww BDE 47 in wild salmon, whereas farmed samples contained BDE 47 at little lower concentrations of 3.1 to 29 μg/kg ww.

Only limited data is available on hexabromocyclododecane (HBCD) concentrations in fish. Concentrations in herring from the Baltic Sea and freshwater locations from Sweden showed considerable concentrations comparable to BDE concentrations. Close to point sources, high concentrations of up to 8 mg/kg lw were detected (Sellstrom *et al.*, 1998). Only recently, data on stereoisomers of HBCD has become available as a result of an isomer specific high performance liquid chromatography-electrospray ionisation-mass spectrometry (LC-ESI-MS) method of analysis (Morris *et al.*, 2003; Tomy *et al.*, 2003).

HBCD has a substantial potential for bioaccumulation. As production continues, it may be expected that HBCD will be found at considerable concentrations in seafood and freshwater fish. Tetrabromobisphenol-A (TBBP-A) data in biota are scarce because laboratories have just established methods for this flame retardant. TBBP-A has the highest production volume of all

Table 21.4 Concentrations of PBDEs and HBCD in fish and shellfish (μg/kg)

Country	Year	Species	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	Sum*	HBCD	lw/ww/dw	References
The Netherlands	1999	Flounder	0.6–20	<0.01–4.6		<0.02-<1				dw	de Boer *et al.* (2003)
		Bream	0.2–130	<0.01–<0.8		<0.9-4.1					
		Mar. Mussel	0.9–4.3	0.3–1.6		<0.1-<0.2					
		Freshw. Mussel	0.7–17	0.4–11		<0.1-1.5					
UK**	?	Eel, trout						4.9–235 (6)	20–3216	ww	Allchin and Morris (2003)
Greenland	?	Sculpin, liver	1.7–7.8							ww	
Sweden, Baltic Sea	2000?	Herring	5.8–21	1.2–8.0	1.1–3.9	0.16–1.2	0.23–1.2		4.3–36	lw	Nylund *et al.* (2001)
Sweden, freshwater***	1995	Pike	40–2000	33–1600	9.3–1000			49–4600 (3)	<50–8000	lw	Sellstrom *et al.* (1998)
Norway	?	Trout, char	0.06–8.3	0.04–8.0						ww	Schlabach *et al.* (2001)
		Burbot, liver	9.7–1044	11–911						ww	
Scotland	1999	Salmon, wild and farmed	15–43	2.8–14	2.4–9.9	ND–1.3	ND–3.6	24.6–85 (9)		lw	Jacobs *et al.* (2001)
Norway			0.4	0.7	ND	ND	ND	1.1			
Ireland	2001	Wild and farmed	5.0–26	1.3–5.6	1.1–7.9	ND	ND–1.3	8.4–52			
			3.1–10	ND–1.6	ND–2.8	ND–0.2	ND	3.1–19			
Japan	1998	Various edible marine fish****	1.2–38	0.13–2.4	0.12–1.5	0.018–1.7	0.21–1.7				Akutsu *et al.* (2001)
USA	1999	Various freshwater fish	1.3–4.0	0.48–0.50	0.44–0.49	0.4–1.0	0.43–1.89	5.3–14 (8)		ww	Rice *et al.* (2002)
Canada, Great Lakes	?	Lake trout	16–58	2.0–14	2.5–5.7	0.89–4.9				ww	Luross *et al.* (2002)
USA, Lake Ontario	?	Sculpin, trout							1–3 α-HBCD 0.3–0.7 γ-HBCD	ww	Tomy *et al.* (2003)

* In brackets the number of summed congeners

** River Skerne, affected by a sewage treatment plant

*** River Viskan, affected by possible point sources

**** Including conger eel, flounder, gray mullet, horse mackerel, red sea bream, sea bass and yellowtail brominated flame retardants. However, due to its more polar character and the covalent binding to plastic in its applications (de Wit, 2002), it will presumably be present at lower concentrations in seafood.

21.3.6 Polychlorinated naphthalenes (PCNs)

PCN mixtures theoretically consist of 75 congeners, although the technical mixtures produced in the past show a different composition due to a different degree of chlorination (Järnberg, 1997). Although individual congeners can be detected, the data reported here are the total PCN concentrations (sum of individual congeners). Typical concentrations determined in fish during the last decade are mentioned in Table 21.5.

Fish from the Baltic Sea and from Sweden showed substantial PCN concentrations. Järnberg found very high concentrations of PCNs up to 360 μg/kg lw in pike assuming that nearby point sources of PCNs contaminated the analysed fish (Järnberg *et al.*, 1997). Baltic fish showed large variation, from 0.54 to 290 μg/kg lw and generally high compared with pg/g-concentrations (ww) found in tuna and swordfish from the Mediterranean (Kannan *et al.*, 2001a). Concentrations in fish from the USA were in the range of 0.019 μg/kg ww (Kannan *et al.*, 2000) to 33 μg/kg ww (Falandysz, 1998).

21.3.7 Polychlorinated terphenyls (PCTs)

Technical PCT mixtures are very complex as theoretically 8557 PCT congeners exist (Remberg *et al.*, 1998). It is not possible to detect individual congeners (as with PCBs) and therefore concentrations of total PCTs are reported (de Boer, 2000b). Literature data must be treated with great care because of the large variation in analytical procedures and calculation methods used.

The available dataset on PCT concentrations in the environment is very limited compared with that on PCBs. Hale *et al.* (1990) determined very high concentrations of PCTs of up to 35 000 μg/kg ww in oysters from the Back River, Chesaspeake Bay, USA, close to a major US aeronautics research facility (see Table 21.6). Much lower concentrations were determined in (shell)fish samples collected from Spanish markets which contained concentrations from *ca.* 1 to 65 μg/kg ww and Bonito Tuna contained concentrations of *ca.* 63 to 260 μg/kg ww (Fernández *et al.*, 1998). Cod liver from the southern North Sea contained concentrations of 410 μg/kg, which were ten times higher compared with the northern part of the North Sea (de Boer, 1995). However, concentrations in twaite shad, herring were considerably lower. Eel caught in Dutch freshwater showed concentrations of 6 to 200 μg/kg ww. The highest concentrations were detected in main river systems such as the rivers Meuse and Rhine. A good relationship could be determined between the concentrations of PCBs and PCTs in biota (expressed on a lipid weight basis), roughly reflecting production volumes of PCBs and PCTs (de Boer, 1995).

Table 21.5 Typical concentrations of PCNs in fish (μg/kg)

Country	Year	Species	lw/ww	Concentration	References
Italy, Mediterranean	1999	Tuna	ww	7.0–22.6*	Kannan *et al.* (2001a)
		Swordfish		14.6–14.8*	
Sweden, freshwater	?	Pike	lw	130–360**	Järnberg *et al.* (1997)
		Pike, Cod, Burbot		2.6–15	
Sweden, Baltic sea		Herring		8.4–26	
Sweden, Baltic Sea	1991–93	Sculpin	lw	0.54–1.5	Lundgren *et al.* (2002)
Poland, Gulf of Gdansk, Baltic Sea	1992	Stickleback	lw	35–130	Falandysz *et al.* (1998)
	?	Fish	lw	8.9–290***	
Baltic sea	?	Herring	lw	0.98–26***	Falandysz (1998)
USA, Purvis Creek	1997	Crab, hepatopancreas	ww	13.3****	Kannan, *et al.* (1998)
		Mullet		6.1	
USA, Great Lakes	1996–97	Various	ww	0.019–26.4	Kannan *et al.* (2000)
USA, Saginaw Bay	?	Fish	lw	0.16–33	Falandysz (1998)

* ng/kg
** Possibly close to point sources
*** Tetra to hepta-CNs
**** Near former chlor-alkali plant

Table 21.6 Typical concentrations of PCTs in fish and shellfish (μg/kg)

Country	Location	Year	Species	Concentration	lw/ww	Standard used	References
The Netherlands	Various freshwater	1991–92	Eel	6–200	ww	A5442	de Boer (1995)
	Hollandse Yssel	1990	Pike perch	5			
	Southern North Sea	1992	Cod, liver	410			
	Northern North Sea			40			
	Southern North Sea	1991	Twaite shad	10			
	Doggersbank	1990	Plaice	1			
	Dutch coast	1991	Herring	3			
USA	Fresh water creek	1987–89	Oyster	400–35 000*	?	A5432	Hale *et al.* (1990)
Spain	Market samples	1996?	Mussel	9.8–18	ww	A5460	Fernández *et al.* (1998)
			Clams	1.2–24			
			Salmon	21–64			
			Tuna (bonito)	63–259			

* Near aerospace complex

21.3.8 Polychlorinated alkanes (PCAs)

PCAs are produced by the chlorination of a mixture of (branched) alkanes with different carbon numbers. In addition a varying degree of chlorination leads to tens of thousands of possible congeners (Muir *et al.*, 2000). The PCAs are subdivided into three groups with different carbon lengths and degrees of chlorination: short chain (SCCA, C10–C13); medium chain (MCCA, C14–C17) and long chain PCAs (C18–C20).

The limited data available is predominantly on concentrations of the SCCAs in fish (see Table 21.7). Generally, concentrations of SCCAs range from *ca.* 100 to 1700 μg/kg lw in fish muscle. Limited data on fish livers from Norwegian burbots show concentrations ranging from 226 to 3700 μg/kg lw.

The limited data hampers clear conclusions on contamination related to species, fat content of the fish and the fishing grounds the fish originates from. Moreover, the available data should be treated with great care for its limited accuracy due to the use of technical mixtures and the detection methods applied (as discussed later).

21.3.9 Organochlorine pesticides (OCPs)

Together with PCBs, OCPs have been analysed for a long time in several fish species. Therefore, a considerable amount of data on the OCPs in fish is available from different species and origin (see Table 21.8). OCPs are included in several national monitoring programmes.

The highest pollution concentrations of HCB, HCHs (sum) and DDTs (sum) are observed in eel from the Netherlands, flounder, perch and mussels from Estonia and Latvia, various fish from the Great Lakes, and also in farmed and wild salmon from Scotland. Fish from the Baltic area show considerable concentrations of α- and γ-HCHs (up to 255 μg/kg ww) and high concentrations of over 1 mg/kg ww of DDE and DDD (Olsson *et al.*, 2000; Roots, 2001). Mostly, DDE is predominant over DDD and DDT. The drin (aldrin, dieldrin) concentrations are generally below 1 μg/kg ww, except for some UK and US Great Lakes fish samples.

The highest concentrations of chlordane (sum) are found in the USA Great Lakes and perch from the Baltic area. With *ca.* nine per cent fat, the herring samples contained *ca.* 5 μg/kg ww chlordane, which is in the same range as fish from North-West European seas. Farmed salmon from Chile contained concentrations of < 0.1 μg/kg ww, whereas concentrations in Icelandic and Norwegian salmon ranged from 1.7—7.6 μg/kg ww (Karl *et al.*, 1998). The concentrations of OCPs in farmed salmon are rather high and are comparable with concentrations in fish originating from polluted waters (Jacobs *et al.*, 2001b). Temporal trends of DDT concentrations are declining (Picer and Picer, 1994; de Vault *et al.*, 1996).

21.3.10 Tris(4-chlorophenyl)methanol (TCPM) and tris(4-chlorophenyl)methane (TCPMe)

TCPM and TCPMe are globally spread contaminants which are found in fish, marine mammals and polar bears (de Boer, 2000c). There is only limited data

Table 21.7 Concentrations of PCAs in fish and shellfish ($\mu g/kg$)

Country	Year	Species	Conc.	Class	lw/ww	References
UK	1979?	Plaice, pouting, pike	<0.05–0.2*		ww	Campbell and McConnell (1980)
		Mussel	0.1–12.0*	C10-20		
		Plaice, pouting, pike	<0.05–0.2*	C20-30		
		Mussel	<0.1–0.1*			
UK	?	Freshwater fish	<100–5200	SCCA**		Nicholls *et al.* (2001)
Marmara Sea		Blue fish	405	C10-13	lw	Coelhan *et al.* (2000)
		Silver side	332			
Mediterranean		Sardine	903			
Atlantic		Angler	206			
		Cod	170			
		Shark	417			
		Sole	207			
North Sea		Herring	805			
Norway	2000?	Trout, Arctic char	108–1692	C10-13	lw	Borgen *et al.* (2001)
		Burbot, liver	226–3700			
USA, Detroit river/Lake Erie	1995	Perch	82	C14-17		Tomy and Stern (1999)
		Catfish	904			

* ppm, mussel close to outlet of PCA production plant

** Chain length not specified

Table 21.8 A selection of typical concentrations of OCPs in fish and shellfish (expressed in μg/kg)

Country	Year	Species	HCB	ΣHCH*	ΣDDT*	ΣCHLs*	Drins	ww/lw/dw	References
Netherlands	1999	Eel	1.2–81	3.5–199** <0.1–66 α <0.9–78 β <4.3–55 γ	11–164 7.2–89 DDE 4.2–62 DDD <0.7–29 DDT			ww	de Boer *et al.* (2000a)
Scotland	1999	Salmon, wild and farmed	13–44	ND–23 ND–6.8 α ND–8.7 β ND–	45–249 29–123 DDE 3.3–46 DDD 8.9–40 DDT			lw	Jacobs *et al.* (2001)
Norway		Salmon, farmed	ND	11	5.1				
Turkey Aegean Sea	1995	Red Mullet			0.86–4.5 DDD 10–18 DDE		0.10–0.61 Aldrin	ww	Kucuksezgin *et al.* (2001)
Greenland	1994–95	Mussel	0.01–0.1	0.2–0.5	0.2–0.8			ww	Dietz *et al.* (2000)
		Arctic char	0.6–1.0	0.4–0.6	2–20			ww	
Greenland, fresh	1994–95	Char	0.72	0.41 0.23 α 0.04 β 0.15 γ	4.0 3.4 DDE 0.13 DDD 0.32 DDT	4.8 0.41 oxy chl. 0.09 tr. chl. 0.54 cis chl. 2.3 tr. nona. 1.2 cis nona.	0.72 Dieldrin	ww	
Ireland	1993–96	Herring				1.5–2.4		ww	Karl *et al.* (1998)
Atlanto-Scandian						1.6–9.7			
South Norway						0.1–1.5			
North Sea						<0.1–5.1			
English Channel						2.2, 3.2			
Farmed Salmon						<0.1–7.6			
West coast UK****	1991–94	Mackerel***	1.3–2.6	0.6–1.8 α 0.6–1.3 γ	5.2–11 DDE		5.4–8.8 Dieldrin	ww	Karl and Lehmann (1997)
Latvia. Lake Burtnieku	1996	Perch	5.9–16	4.1–8.8 α 7.4–22 γ	40–1000 DDE 13–140 DDD	2.0–16 tr. nona.		ww	Olsson *et al.* (2000)

Table 21.8 Continued

Country	Year	Species	HCB	ΣHCH*	ΣDDT*	ΣCHLs*	Drins	ww/lw/dw	References
Estonia, marine	1998	Flounder		2.3–112 α 6.1–255 γ	10.1–1521 DDE 45–4130 DDD 14–47 DDT			lw	Roots (2001)
		Perch		2.1–31 α 2.2–74 γ	22–119 DDE 0.4–51 DDD 1.2–50 DDT				
	1998	Mussel		6.7–8.5 α 4.3–4.8 γ	45.8–74 DDE 7.4–13 DDD 24–44 DDT				
West-Baltic Sea	1990–91	Mussel	0.2–2.4	2.5–22	6.6–88 3.9–40 DDE 0.9–29 DDD 1.8–18 DDT			dw	Lee *et al.* (1996)
Southern Baltic	1991–93	Herring				49 6.0 oxy chl. 2.3 tr. chl. 14 cis chl. 12 tr. nona. 3.6 cis nona.		lw	Strandberg *et al.* (1998)
Alaska	?	Trout	0.46	0.56	2.7	1		ww	Allen-Gil *et al.* (1997)
USA, Great Lakes	?	Various commercial fish	0.22–9.3	0.28–23	1.1–124	0.94–45	0.24–41 Dieldrin	ww	Newsome and Andrews (1993)
Canada, Northwater Polynya	1998	Arctic cod		90 40 α 27 β 23 γ				ww	Moisey *et al.* (2001)

* Total concentrations. Concentrations of individual compounds specified if available

** Highest concentration originates from a polluted area close to a former lindane production site

*** Length class mentioned: 37–38 cm

**** Concentrations in mackerel from German Bight, from Norwegian coast and close to Shetlands were in the same range

Table 21.9 Concentrations of TCPMe and TCPM in fish and shellfish (μg/kg lipid weight)

Country	Location	Year	Species	TCPM	TCPMe	Reference
The Netherlands	Northern North Sea	1992	Cod liver	5.7	1.7	de Boer (1997)
	Southern North Sea	1993		40	ND	
	Southern North Sea	1987	Cod liver oil	51	3	
	Southern North Sea	1987	Mackerel oil	35	0.6	
	Wadden Sea	1994	Mussels	13	<6	
	Freshwater	1994	Eel	10–360	ND–37	
Italy?	Mediterranean Sea	1992	Tuna, perch, angler, mullet	<1	<1	
Poland	Baltic South coast	1991–95	Various	ND–11	ND–30	Falandysz *et al.* (1999)

available (see Table 21.9) on concentrations of TCPM and TCPMe in fish. TCPMe shows generally lower concentrations (factor 2 to 50) compared with TCPM, although samples from the Baltic Sea contained higher concentrations of TCPMe (up to 30 μg/kg lw) than TCPM (up to 11 μg/kg lw) (Falandysz *et al.*, 1999).

The only freshwater fish with relevant data reported in the literature was eel from different freshwater locations from the Netherlands showing concentrations up to 360 μg/kg. Due to the limited data on toxicity of these compounds (de Boer, 2000c) and the absence of fish data from other than European regions it is not possible to draw conclusions on the impact on human health of these contaminants.

21.3.11 Toxaphene

Although toxaphene consists theoretically of over 30 000 congeners, in environmental samples only a few hundred congeners are present. Initial reports were based on total toxaphene, but the increasing commercial availability of individual congeners and information on the persistency of specific congeners led to the determination of a selection of individual congeners (de Geus *et al.*, 1999; Whittle *et al.*, 2000; Vetter *et al.*, 2001).

A selection of typical toxaphene concentrations is shown in Table 21.10. The Great Lakes area in North America, known for their contamination with toxaphene, show considerably high concentrations, up to almost 2 mg/kg ww total toxaphene. The tolerable daily intake (TDI) of toxaphene, estimated to be 0.2 μg/kg/day can only be exceeded by regular consumers of highly contaminated fish (Berti *et al.*, 1998). In 1997 a European research project titled 'Investigation into the Monitoring, Analysis and Toxicity of Toxaphene (MATT)' was initiated. As a part of this programme, concentrations of the individual congeners Parlar no. 26, 50 and 62 were determined in a vast amount of samples in marine and freshwater fish in north-

Table 21.10 Concentrations of toxaphene in fish and shellfish (μg/kg wet weight)

Location	Year	Species	Individual congeners						Total Tox. (mg/kg ww)	References
			P26	P40	P41	P44	P50	P62		
Iceland	2000?	Cod, liver	10–18	6–9	3–4	20–35	11–12	10–14		Vetter *et al.* (2001)
Baltic Sea		Cod, liver	35–83	3–16	6–17	31–88	21–40	8–41		
Baltic Sea, Swedish coast		Salmon	2.22–10.5				2.8–16	0.39–3.0		Atuma *et al.* (2000)
Greenland	1994–95	Arctic char	0.67				1.8	0.64	0.013	Cleemann *et al.* (2000)
USA, Lake Michigan	1997–98	Various							0.15–1.2	Stapleton *et al.* (2001)
USA, Lake Superior	1998	Lake Trout	0.02	0.02	0.01	0.02	0.11	0.06	1.9	Whittle *et al.* (2000)
USA, Lower Rio Grande Valley	1997	Common carp							ND–0.31	Wainwright *et al.* (2001)

west European samples and arctic samples. Due to the global distillation effect, concentrations are higher in the colder regions (e.g. Barents Sea) compared to, for example, the Netherlands (de Boer *et al.*, 2004). The predominant congener contribution to the sum of P 26, 50 and 62 varies with the origin of the samples.

21.3.12 Nonylphenol (ethoxylate) compounds

Most of the reported data originates from fish caught in waters in industrialised/urbanised areas. Some of these waters may be influenced by sewage treatment plants (Vethaak *et al.*, 2002). No data is reported on fish from remote areas like the arctic regions. The data ranges from 5 μg/kg ww to 380 μg/kg dw for NP and up to 3100 μg/kg dw for its ethoxylates (see Table 21.11). The highest concentrations reported were detected in fish from the Glatt River in Switzerland, which is influenced by one nearby sewage treatment plant (Ahel *et al.*, 1993). The concentrations in fish from marine waters are relatively low.

Alkylphenols are known for their estrogenic potential (Odum *et al.*, 1997; Vethaak *et al.*, 2002). Although the bioaccumulative properties of alkylphenols are less strong than, for example, those of PCBs, the combination of the concentrations found and the estrogenic effects cause concerns about the occurrence of these compounds in fish.

21.3.13 Musk compounds

After their application in private households, musk compounds are dumped in the environment via sewage treatment plants. This is the main route for contamination of (freshwater) fish, which is shown by the high concentrations of musk compounds (up to 20.3 mg/kg lw for 1,3,4,6,7,8–hexahydro-4,6,6,7,8,8, hexamethylcyclopenta(g)-2–benzopyrane (HHCB)) in rainbow trout that originates from the River Stör, 3 km downstream of a sewage treatment plant (Table 21.12).

Eel samples from the Netherlands contained concentrations of musk xylene (MX) and musk ketone (MK) in the range of <0.5 to 45 μg/kg ww, with MX being predominant (de Boer and Webster, 1999). This is in the same range as observed PCB concentrations in similar samples. No data on HHCB and 7–acetyl-1,1,3,4,4,6, hexamethyltetrahydronaphthalene (AHTN) have been reported for Dutch samples. Data from German fish samples show that the polycyclic musk compounds are predominant compared with the nitro musks, which reflects the higher production and use figures discussed earlier (Rimkus, 1999). On the contrary, Canadian clam samples from Halifax harbour showed the highest concentrations of MK (Gatermann *et al.*, 1999).

21.3.14 Perfluorinated compounds (PFCs)

PFCs is a relatively new group of substances. Very little is known on the toxicity and fate of PFCs. Physicochemical properties and behaviour cannot be predicted

Table 21.11 Concentrations of nonylphenol (ethoxylates) in fish ($\mu g/kg$)

Country	Year	Species	NP	NP1EO	NP2EO	dw/lw/ww	References
Switzerland	1984–85	Various fish	150–380	180–3100	130–2300	dw	Ahel *et al.* (1993)
USA, Lake Mead	1999	Carp	184	242		ww	Snyder *et al.* (2001b)
UK, River Tyne	1997	Flounder	5–55	<LOD		ww	Lye *et al.* (1999)
Italy, Orbetello lagoon	1998–99	Grass goby	0.12–1.6	28–508	0.3–48	ww	Corsi and Focardi (2002)
The Netherlands, various freshwater locations	1999–2001	Bream	30–160	150–500		ww	Vethaak *et al.* (2002)
The Netherlands, Wadden Sea		Flounder		100			
The Netherlands, harbour	1996	Flounder, liver	< 150			ww	de Boer *et al.* (2001b)

Table 21.12 Typical concentrations of musk compounds in fish and shellfish (μg/kg)

Country	Location	Year	Species	M-xylene	M-ketone	HHCB	AHTN	lw/ww/dw	References
The Netherlands	Meuse, Eijsden	1996	Eel	45	11			ww	de Boer and Wester (1996)
	Various			<3–17	4–33				
			Pike perch	<0.5	<0.5				
	Southern North Sea		Various	<2	<4				
			Shrimp, mussel	<0.5	<1				
Germany	Berlin	1996	Eel	1–79	1–380	15–4131	10–1408	ww	Fromme *et al.* (1999)
	River Ruhr	?				400, 600	500, 700	lw	Rimkus (1999)
	River Elbe	?	Pike perch	<10–90	10–70	600–3840	320–990		
	River Stor*	?	Brown trout	200, 240	1000, 1200	13700, 20300	10600, 13400		
North Sea		?	Shrimps	<10–10	20–430	<40–60	<40–60		
North Sea coast		?	Mussels	<10–20	10–30	<30–110	<30–60		
Canada		?	Various	ND–0.8	ND–140	ND–27	ND–6	ww	Gatermann *et al.* (1999)

* 3 km downstream a sewage treatment plant

Table 21.13 Concentrations of PFFs in fish and shellfish ($\mu g/kg$ wet weight)

Country	Location	Year	Species	PFOS	PFOA	lw/ww/dw	References
USA	Michigan waters	1990s	Carp, Chinook salmon, brown trout and lake whitefish	7–300		ww	Giesy and Kannan (2001)
USA	Gulf of Mexico and Chesapeake Bay	1996–98	Oysters	<42–1225		dw	Kannan *et al.* (2002)
Belgium	Western Scheldt/North Sea	2001	Crab, shrimp	25–800		ww	van de Vijver *et al.* (2002)
Belgium	Western Scheldt/North Sea	2001	Plaice, bib	<10–111		ww	Hoff *et al.* (2003)
Canada	Toronto	2000	Common shiner, liver	2000-72 900*	6-91*	ww	Moody *et al.* (2002)
Japan	Tokyo Bay	2002	Var. fish, liver	3–7900		ww	Taniyasu *et al.* (2003)

* After accidental release of fire retardant foam

using models for lipophilic substances like PCBs as these compounds have both hydrophobic and oleophobic type properties and form a third phase in an octanol-water partitioning system which is normally used to predict lipophilicity (Moody and Field, 2000). PFCs have been applied for their water and fat repelling properties in, for example, coatings for cardboard food containers, in dirt repelling coatings for shoes, carpets and leather, and in aqueous fire-fighting foams. Some PFCs are used as a polymerisation aid in the production of Teflon.

Although limited data is currently available, it is expected that more information will become available on concentrations of PFOS and other fluorinated substances in fish in the near future. A selection of current data is given in Table 21.13. High concentrations of PFOS were found in fish and shellfish from the United States, up to 1225 μg/kg dw (Kannan *et al.*, 2002). High concentrations of PFOS were also found in Belgium (North Sea and Western Scheldt estuary) up to 800 μg/kg ww PFOS. Perfluorooctanoic acid (PFOA) and homologues of these surfactants were also determined in freshwater fish after an accidental release of fire-fighting foams (Moody *et al.*, 2002) (nd-2210 μg/l). Long chain PFCs (other than PFOS) were also determined in biota from the Canadian arctic at levels from <0.5 to 230 μg/kg ww in fish livers.

These data show that PFOS is distributed on a global scale. Furthermore, other PFCs have also been detected in fish from various (remote) locations, although at lower levels compared to PFOS. Considering the lack of knowledge on the physical behaviour, toxicity, bioaccumulation and fate of these compounds on the one hand, and the high levels, global distribution and production volumes on the other, there is an urgent need for more research in the coming years.

21.4 Analysing particular organic contaminants in fish

21.4.1 Analysis of PCBs

The general procedure for analysis of lipophilic organic substances consists of the following basic steps: extraction; clean up and final determination of the compounds. Figure 21.1 shows the general scheme for the analysis of PCBs and other lipophilic compounds discussed in this chapter.

Extraction

PCBs are lipophilic, so the extraction methods are based on the isolation of the lipid fraction from the sample matrix (de Boer, 2001). Soxhlet extraction has been used for the extraction of PCBs from a wide variety of matrices including fish using mixtures of non- and medium-polar solvents (e.g. n-pentane/dichloromethane, 1:1, hexane/acetone, 1:1). The non-polar solvent extracts the PCBs from the triglycerides, the medium-polar solvent extracts the PCBs from the phospholipids or other more polar solvents (Hess *et al.*, 1995). Prior to the extraction the sample is macerated, homogenised and ground with dry sodium sulphate to bind the water present in the sample. Freeze-drying is not

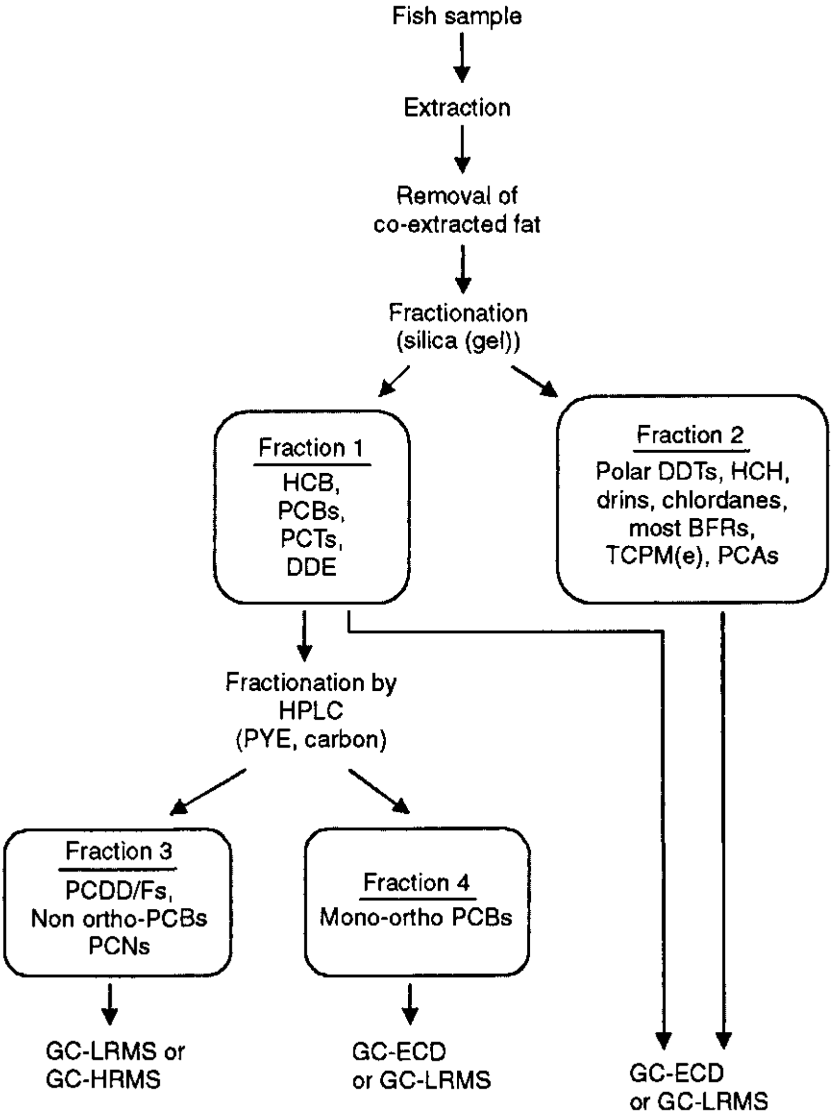

Fig. 21.1 General analysis scheme for the lipophilic organohalogen contaminants discussed in this chapter.

recommended as it may easily cause losses of the more volatile PCBs and may cause cross-contamination. Other extraction techniques used are supercritical fluid extraction (SFE), solid-phase extraction (SPE), accelerated solvent extraction (ASE), microwave assisted extraction (MAE) and cold extraction by Ultra-Turrax (de Boer, 2001).

Clean up

The clean up of the extracted lipid fraction consists of the removal of the lipids from the extract and fractionation in order to remove possible interfering compounds. The lipid removal is carried out by destructive methods (saponification of sulphuric acid treatment) or by non-destructive methods such as gel permeation chromatography (GPC) or alumina columns, with the latter being favoured over GPC for its efficient lipid removal (Hess *et al.*, 1995; de Boer, 2001). However, rigid gels are nowadays available for high performance GPC which result in improved separation. Subsequently, pre-fractionation is carried out in order to separate the PCBs from other compounds like organochlorine pesticides in order to reduce possible interferences during gas chromatographic separation of the PCBs. Silica gel columns or Florisil columns are frequently used and the process can be automated (Hess *et al.*, 1995; de Boer, 2001).

Determination

PCBs are a complex mixture, consisting of 209 different congeners. The method of choice is capillary gas chromatography (GC) since this technique enables the determination of individual compounds. The separation is carried out on capillary columns with a stationary phase that is providing enough selectivity. Stationary phases with varying polarity are used, such as CP-Sil-8 (95% methyl, 5% phenylpolysiloxane) or CP-Sil-19 (85% methyl, 7% phenyl, 7% cyanopropyl, 1% vinylpolysiloxane) providing different resolution, but no single column is able to separate all 209 congeners (de Boer *et al.*, 1992; Hess *et al.*, 1995; de Boer, 2001). Narrow bore columns (0.15 mm internal diameter) are preferred for their increased resolving power (de Boer and Dao, 1989). Multi-dimensional GC techniques may become more important in the near future for PCBs and complex mixtures of contaminants in general, as with that technique peak overlap can be almost completely avoided (Korytar *et al.*, 2002). Figure 21.2 shows the potential of comprehensive multi-dimensional GC (MDGC) by a nearly complete separation of a standard mixture of PCBs and WHO dioxins and furans. Electron capture detectors (ECD) and modern bench-top mass spectrometric detectors (MSD) are used, the latter being more selective than the ECD (Hess *et al.*, 1995; de Boer, 2001). DI-PCBs require a more specific clean up, comparable to that of dioxins. They can be determined by low resolution MS.

21.4.2 Analysis of PCDDs, PCDFs and non-ortho substituted PCBs

The concentrations of dioxins and furans in samples occur at a 100–1,000-fold lower concentration than PCBs and therefore a high sensitivity and lower limits of detection are required (Liem, 1999). The analytical procedure should be highly selective as a distinction is required for dioxins, furans and dioxin-like PCBs from a multitude of other, co-extracted and possibly interfering compounds present at concentrations up to several orders of magnitude higher than the compounds of interest (Liem, 1999).

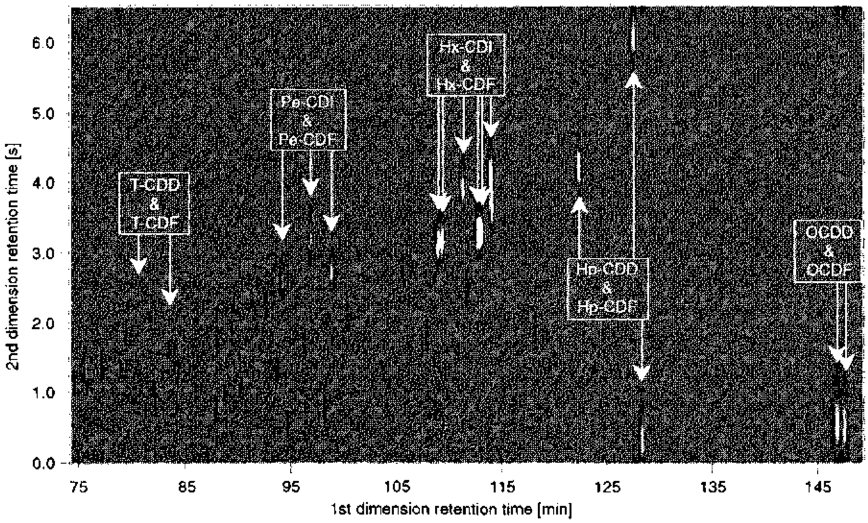

Fig. 21.2 GCxGC-μECD analysis of a mixture of 90 PCBs and 17 WHO PCDD/Fs with a HP-1-HT-8 column combination. Reprinted with permission from Korytar *et al.* (2002).

The pre-treatment and extraction procedure, as well as the removal of the lipids from the extract, is very similar to the PCB analytical procedure. Subsequently, the extract is applied to an additional fractionation step, carbon or PYE column (2-(1-pyrenyl)ethyldimethylsilyl) chromatography, retaining planar multi-ring compounds like non-ortho PCBs, PCDDs, PCDFs as well as other planar compounds (Hess *et al.*, 1995; Liem, 1999).

The final determination is carried out by GC-high resolution MS (GC-HRMS), which currently is the only technique able to provide the required selectivity and sensitivity (Liem and Theelen, 1997). However, currently instrumental techniques (GCxGC-ECD, GC-ion trap low resolution MS/MS) are being developed that might be able to provide the required selectivity and sensitivity (www.dioxins.nl).

21.4.3 Analysis of other contaminants

The PAH analysis is fairly straightforward and generally starts with alkaline saponification of the lipids and subsequent extraction of the PAHs with an organic solvent (Baumard *et al.*, 1998; Kelly *et al.*, 2000). The extract is further cleaned by alumina, silica gel or aminosilane chromatography (Baumard *et al.*, 1998; Kelly *et al.*, 2000; Piccardo *et al.*, 2001). The cleaned extract can be analysed by HPLC, using PAH tailor-made columns (establishing pi-pi interactions with the PAHs) and subsequent fluorescence detection (Kelly *et al.*, 2000; Piccardo *et al.*, 2001). On the other hand, GC-EI-MS is emerging for PAH analysis using apolar columns like HP-5 or CP-Sil 5 (Baumard *et al.*, 1998; Kelly *et al.*, 2000; Baussant *et al.*, 2001). GC-MS has specific advantages like the ability to detect non-fluorescent PAHs which cannot be analysed by HPLC-fluorescence detection, although both techniques are complementary to each other. Deuterated compounds are commercially available which can be used as internal standards in GC-MS quantification (Kelly *et al.*, 2000).

As shown in Fig. 21.1, most other lipophilic organohalogen contaminants can be determined using comparable extraction and clean-up strategies as being used for the analysis of PCBs and PCDD/Fs. However, specific adaptations are required for each group of contaminants depending on the complexity of the technical mixture (e.g. PCAs and PCTs), the possible thermal or chemical degradation of some contaminants (e.g. some OCPs and decaBDE) or the unknown behaviour during extraction and clean up (e.g. PFCs).

PBDEs, TBBP-A and HBCD can be determined by GC-ECD and GC-MS, although MS is preferred because use can be made of the prominent bromine clusters in the MS spectra. The fully brominated diphenyl ether (deca-BDE) is sensitive to deterioration by UV light and thermal heating in the GC oven and therefore measures should be taken to prevent losses of deca-BDE during analysis. De Boer *et al.*, (2001a) determine deca-BDE separately on a shorter column (15 m) to minimise deterioration of this thermo-labile compound. Recently, the individual stereoisomers of HBCD have also been analysed by HPLC-ESI-MS (Morris *et al.*, 2003).

Considering only 75 possible congeners, the gas chromatographic analysis of PCNs is fairly straightforward using non-polar columns like Ultra-1 which was able to separate 44 in a Halowax 1014 mixture, out of 75 possible congeners (Järnberg *et al.*, 1994). Detection is performed by NCI or EI-(HR)MS in the SIM mode, with electron ionisation (EI) being favoured as the responses are similar for all congeners (Jakobsson and Asplund, 2000).

The technical mixture of PCTs is very complex (8557 theoretical congeners). PCTs are found in the same fraction after silica gel elution as PCBs. Higher temperatures are required to elute them from GC columns because they have higher boiling points. Total determinations, using the technical Aroclor mixtures as standards, have been used (Wester *et al.*, 1996) as congener-specific determination is still not possible. Comprehensive MDGC may be a solution for this problem in the future.

Technical PCA mixtures consist of tens of thousands of congeners and separation of all individual congeners is impossible by single column GC and probably even not possible with multi-dimensional GC. Therefore, methods are developed that result in the sum of short-, medium- of long chain-CAs, based on comparison with one of the technical mixtures available (Muir *et al.*, 2000). The use of technical mixtures that do not match the pattern as observed in the sample decrease the quality of data reported (Coelhan *et al.*, 2000) and therefore data should be treated as semi-quantitative.

The methods for OCP determinations are similar to those for PCB analysis. Traditional methods for PCB and OCP clean up result in two fractions after the elution over the silica column (see section PCBs), one PCB fraction and one OCP fraction in which most pesticides are present. However, (a part of) trans-nonachlor, cis- and trans-chlordane, hexachlorobutadiene (HCBD), QCB, HCB, OCS, p,p-DDE, o,p-DDE, PCA and PCTA may be found among the PCBs in the first fraction as well. Some OCPs (e.g. dieldrin, endrin and p,p-DDE) decompose under sulphuric acid and therefore, this clean-up step should be avoided when analysing these specific OCPs (de Boer and Law, 2003).

For TCPM and TCPMe, the extraction and clean-up procedure is similar to the OCP procedure. TCPM and TCPMe elute at relatively high oven temperatures (270—300 °C), which is comparable with the PCTs. Details on the analysis of these compounds can be found in a review by de Boer (2000c).

The very complex mixture of toxaphene, consisting of theoretically 32,768 possible congeners disables the complete separation of all individual congeners (de Geus, 2000). With the application of multi-dimensional GC an increased separation power was obtained resulting in 246 peaks (more than twice the number obtained in single column GC) (de Boer *et al.*, 1997). For the quantification of toxaphene often technical mixtures and levels are reported as total-toxaphene resulting in a possible mismatch between the congener profile present in the sample and the technical mixture (Vetter and Oehme, 2000; Breackevelt *et al.*, 2001). Although there is a lack of standards, a limited number (mixtures from) of individual congeners (e.g. Parlar nos. 26, 32, 40, 41, 44, 50 and 62) are commercially available (Vetter and Oehme, 2000). For this reason (a selection of) these individual congeners are generally reported.

The analysis of musk compounds is very similar to the methods applied for PCBs and OCPs, generally starting with (Soxhlet) extraction of the lipids from the sample, subsequent lipid removal by aluminum oxide or GPC (Fromme *et al.*, 1999; Rimkus, 1999). The PCBs and OCPs are removed from the extract by separation on silica gel column. The final determination is carried out by GC-ECD, GC-NPD, GC-EI-MS or GC-NCI-MS, whose specific (dis)advantages are discussed by Rimkus (1999).

Nonylphenol and short-chain nonylphenol ethoxylates (NP1EO and NP2EO) in fish can be analysed by both HPLC and GC. The analysis starts with extraction of the compounds from the matrix by Soxhlet extraction (Snyder *et al.*, 2001b) or steam distillation (Ahel *et al.*, 1993; Lye *et al.*, 1999; Snyder *et al.*, 2001), followed by GPC, alumina chromatography or silica chromatography for removal of the lipids (Lye *et al.*, 1999; Snyder *et al.*, 2001b; Tsuda *et al.*, 2001). Quantification is fairly straightforward and is performed by normal-phase HPLC and fluorescence detection (Ahel *et al.*, 1993; Snyder *et al.*, 2001b). GC-MS is also frequently employed for detection and quantification (Lye *et al.*, 1999; Snyder *et al.*, 2001b; Tsuda *et al.*, 2001).

The analysis of PFCs is not as 'straightforward' as the already complicated analysis for PCBs or PBDEs. As PFCs have surfactant properties, the behaviour of PFOS during sample treatment or determination is not yet completely understood, which makes the analysis susceptible to analytical mistakes and problems. Giesy and Kannan (2001) extracted biota samples after addition of water and a counterion and bufferd it at pH 10, following extraction with organic solvent (methyl-tert-butyl-ether). The separation was performed on a C18 HPLC column and detection was performed by electrospray(ESI)-MS (negative ion mode). Recoveries range typically from below 50% to over 150% showing that the method of analysis is not yet completely under control (Giesy and Kannan, 2001; Kannan *et al.*, 2001b, 2002; Taniyasu *et al.*, 2002; Hoff *et al.*, 2003). Moody *et al.* (2001) used a similar HPLC-ESI-MS method for determination of PFOS and related substances in water samples (Moody *et al.*, 2001). Derivatising and determination with GC has limited utility (Moody and Field, 2000).

21.5 Health issues for organic contaminants in fish

The toxic potency of the substances discussed varies considerably among the different contaminant groups. Dioxins have shown developmental and reproductive effects in rats and monkeys and have carcinogenic potency (Liem and Theelen, 1997; SCF, 2000). Within the EU a tolerable weekly intake (TWI) of 14 pg WHO-TEQ/kg bodyweight (bw)/week was advised by the EU Scientific Committee for Food (SCF). Fish generally shows highest concentrations of dioxins among other food items and therefore, humans with diets including a substantial amount of fish (Scandinavia, Southern European countries) have an increased chance to exceed the TWI of 14 pg PCDD/F-TEQ/kg bw/week. A new set of European maximum residue limit (MRL) values for food including fish and fish oil (4 pg PCDD/F-TEQ/g ww and 2 pg PCDD/F-TEQ/g fat, respectively) (Anon., 2001) became active on 1 July 2002. From Table 21.3 it is clear that only a limited amount of fish and shellfish samples exceeded the limit 4 pg PCDD/F-TEQ/g ww. It is anticipated that in 2004 the MRLs will be evaluated in the light of possible inclusion of the dl-PCBs.

PCBs, other than the dl-PCBs, have a tumour promotion potential (independent of the dioxin-type of toxicity) (van der Plas, 2000). Based on this observation, tolerable daily intake (TDI) of 300 ng/kg bw/day was calculated. Depending on the composition of the diet, this TDI can possibly be exceeded for consumers with fish rich diets or when highly contaminated fish is consumed. For PCBs several countries have limit values for the sum of indicator PCBs or for individual congeners. The Netherlands has congener specific limits up to 600 μg/kg PCB 153 in eel, whereas in Belgium currently a limit of 75 μg/kg for the sum of the indicator PCBs is discussed. The later limit could lead to considerable non-compliance for freshwater fish and some non compliance for marine fish.

Concerning toxaphene, Germany and Austria have set a MRL (0.1 mg/kg ww for three indicator congeners and the sum of toxaphene, respectively). Table 21.10 shows that samples from the Great Lakes exceed the total toxaphene MRL of Austria, whereas none of the samples from Germany, the Netherlands, Sweden and Ireland exceeded the MRL (de Boer *et al.*, 2004). The United States and Canada have set a TDI .

The toxicity of NP is not extensively investigated. However, estrogenic activity of NP was observed from an induction of a uterotropic response in rodents after exposure to NP (Odum *et al.*, 1997). It is not clear how these results should be interpreted for disrupting human hormonal systems and the impact on human health.

For some substances like PFOS and PCAs, very limited or no data is available on human toxicity and therefore the relevance cannot be expressed. However, based on the production volumes and use (in the past), there is some concern on the possible toxic potency of these substances.

In normal risk assessment approaches, the health effects of individual contaminants or a group of contaminants are evaluated. For example the MRLs for the WHO-PCDD/Fs are based on this approach (van den Berg *et al.*, 1998; EC, 2001). In a recent study on contaminants in farmed salmon samples, consumption advice was developed based on potential cancer risks and on the assumption of a risk additivity of dieldrin, toxaphene and PCBs. This resulted in strict recommendation of consumption of between less than one (European farmed salmon) to eight meals of (North America farmed) salmon per month (Hites *et al.*, 2004).

21.6 Future trends

A vast amount of data has been produced during the last two decades on concentrations of a wide range of contaminants in fish. Monitoring data show that concentrations of PCBs and OCPs are declining and, except for some hot spots, concentrations are generally below the applicable MRLs. This is mainly due to phasing out of the use of PCBs and OCPs and the controlled way of dismantling old transformers in order to prevent PCB-containing oils from entering the environment. Also other contaminants like PCNs, PCTs and toxaphene are not used any more and it is expected that concentrations are declining, although less research has been conducted for these contaminants, and monitoring data are not available.

BFR concentrations in fish reflect the ongoing production of these chemicals. Although the penta-mix related PBDEs show a decline in Europe (but not in the United States (de Wit, 2002)), other relatively new substances like HBCD and TBBP-A show considerable concentrations in fish. A temporal trend studied in guillemot samples shows an increasing trend from 1969 to 1997 (de Wit 2002). Furthermore, the toxicological importance of these compounds needs further research efforts. This is currently being assessed in several research studies in Europe.

Other contaminants still in use but with very little data are alkylphenols and their ethoxylates, musk compounds and PCAs. Future monitoring should also focus on these substances. Furthermore, PFOS and other fluorinated substances represent a group of contaminants of which very little is known. The unique properties (compared to lipophilic contaminants) hamper the prediction of concentrations in the environment, fate, toxicity and metabolism. Scientists should pay attention to this class of substances to fill the data gaps in order to evaluate environmental concentrations and health risks of these contaminants.

Recent crises on dioxins and BSE in food (Belgium, 1999) have generated consumer concern about undesired substances in their food. As a result, research and monitoring of contaminants in food in the European Union have intensified. It is therefore expected that more data will become available on contaminants in fish for legislative purposes and to evaluate positive and negative health aspects of fish consumption.

The farming of fish, of which volumes are increasing world-wide (FAO 2001), provides the possibility to control the concentrations of contaminants in the produced fish. Fish meal and fish oil from Chile and Peru show considerably lower concentrations of dioxins (SCAN, 2000) and therefore, by careful selection of these little contaminated ingredients for farmed fish diets the concentrations in the farmed fish can be kept low. Furthermore, the replacement of fish oil and fish meal by vegetable ingredients provides another possibility to reduce organic contaminant concentrations in farmed fish.

21.7 Sources of further information and advice

This chapter has focused mainly on concentrations of contaminants in fish. Information on other aspects of these contaminants like concentrations in other environmental compartments, toxicity research and evaluation, human intake and legislative aspects are not discussed within this framework. Some recent and extensive reviews on these subjects are mentioned below:

J. Paasvirta (2000), New Types of Persistent Halogenated Compounds. Springer-Verlag. 3, part K, Berlin, Germany. Contaminants discussed are: TCPM and TCPMe, PCTs, PBDEs, PCNs, PCAs and toxaphene.
G. Rimkus, Synthetic Musks in the Environment. Elsevier, Amsterdam, The Netherlands, in press.
Liem, A. K. D. and R. M. C. Theelen, 1997, Dioxins: chemical analysis, exposure and risk assessment, Thesis, University of Utrecht, The Netherlands

Information concerning legislation, safety and current monitoring can be obtained from the following websites:

www.epa.gov
www.who.int
www.europa.eu.int
www.efsa.eu.int

21.8 References

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