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Distribution of Persistent Lipophilic Contaminants in Fish from the Grand Duchy of Luxembourg

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Due to their wide spectrum of chemical structures and physical characteristics organochlorinated compounds (OCs) have been largely used in industry (PCBs), in agriculture (DDT, dieldrin, lindane) or in both sectors (hexachlorobenzene HCB, pentachlorohenol). Some OCs are side products of manufacturing processes (δ-HCH in lindane, *o,p'*-congeners of DDT) or are metabolites (DDD and DDE of DDT, Table 1, Smith 1991, WHO 1993). Synthetic musk derivatives (such as musk xylene, MX) are added to cosmetics, soap and washing powder (Käfferlein et al. 1998). Musk derivatives are not halogenated, but their nitro groups and their lipophilicity make it possible to extract and detect them together with OCs. Due to their persistence, OCs and MX became notorious as environmental contaminants ever since 1966 (PCBs, Jensen 1966) and 1981 (MX, Yamagishi et al. 1981). The use of OCs has been limited in industrialized countries or they have been completely withdrawn from the market (WHO 1993). OCs are not acute poisons, but there have been many discussions on their chronic toxicity. In commercial mixtures, highly toxic coplanar PCB congeners and impurities such as dioxins may be present. Many known biological effects of these toxicants are thought to be mediated through the Ah receptor (Poland and Knutson 1982), but non-Ah-receptor-mediated mechanisms have been discussed as well (Ahlborg et al. 1992b, Machala et al. 1998). Concerns on possible interactions in hormonal regulation mechanisms recently fuelled the discussion on the chronic toxicity of OCs (Beard et al. 1999, Gerhard et al. 1998, Sonnenschein and Soto 1998). Previous measurements had shown that PCBs in fish from the Grand Duchy of Luxembourg exceeded the limit allowing a long term survival of the otter (Hugla et al. 1998). The aim of this study was to measure OCs and MX in order to assess the human intake when consuming local fish and to elucidate how these contaminants are distributed in the Grand Duchy of Luxembourg.

MATERIALS AND METHODS

Fish species frequently consumed in Luxembourg were captured with an electrical pole in June 98 and April 99 in the following rivers (Fig. 1): Sûre (5 capturing sites: Sûre1-5), Alzette (Alz), Ernz blanche (Ebl), Ernz noire (Enoi), Our (2 capturing sites: Our1&2), Syr, Moselle (Mos). Guidelines concerning species, size, capturing season and number of individuals elaborated by the International Commissions for the Protection of the Moselle and Sarre for monitoring the contamination by fish analyses were considered as far as possible (CIPMS 1994a).

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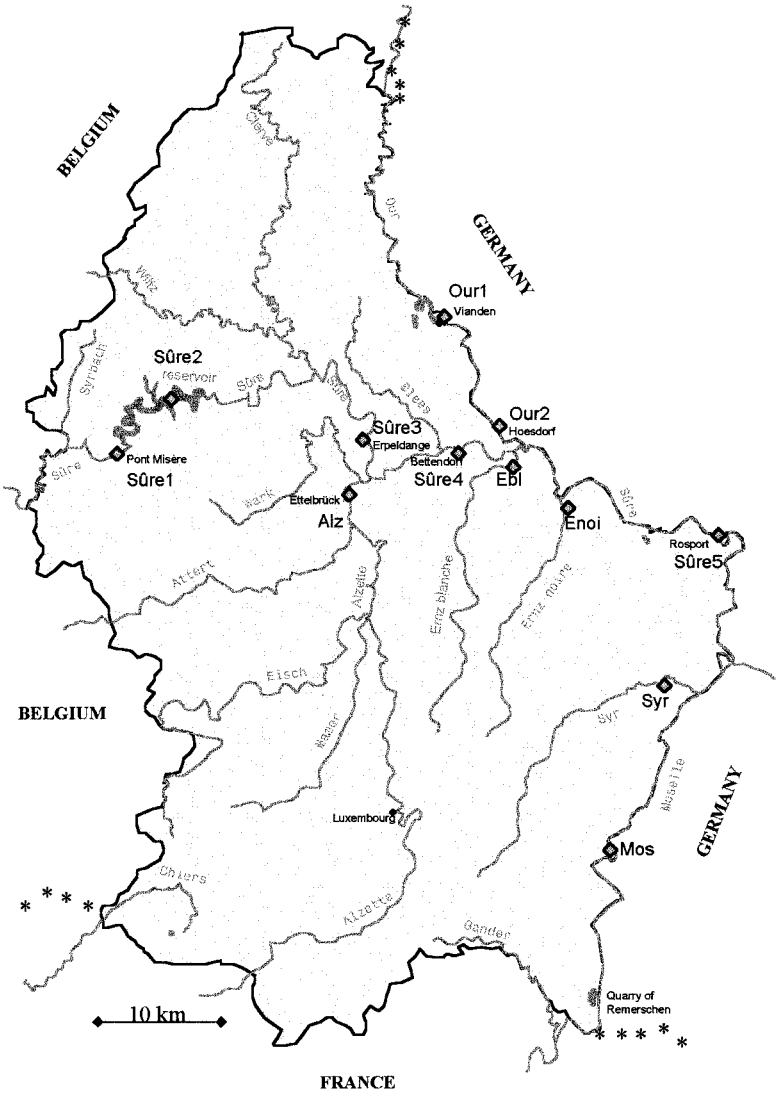

Figure 1. Grand Duchy of Luxembourg and sites where fish were captured.

The fish were brought to the laboratory, measured, weighed, and dissected. Visible eggs were used to determine female sex. Skin tissue was scaled before determining fat content. The dissected tissue was frozen at –20°C until analysis. Muscle tissue was analyzed predominantly (human consumption). For discussion and for comparison with literature data, some juvenile fish as well as skin and eggs of some adult fish were also analyzed (Table 3).

References (Table 1) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Cartridges, frits, EnvirElut® sorbent and Florisil columns (500 mg) for subsequent clean-up were obtained from Varian (Zaventem, Belgium). One nanogram of PCB 209 was added to 0.05 to 0.5 g fish fresh wet weight tissue (amount depending on the expected fat content, approximately 10 mg lipids were needed) and blended in a mortar with four times the fish weight of EnvirElut® sorbent until homogeneous. The mixture was transferred into an empty 15 mL cartridge and covered with a frit. The lipids were extracted 2 times with 3 mL dichloromethane. The eluated solvent was evaporated to dryness under a stream of nitrogen at 40°C. The extracted lipids were determined gravimetrically and expressed as per cent of wet weight. One mL of petroleumbenzene was added to the extract which was poured on a Florisil column previously rinsed with petroleumbenzene:dichloromethane (8:2, v/v). The column was rinsed 2 times with 3 mL petroleumbenzene:dichloromethane (8:2). The eluated solvent was carefully evaporated, the extract was reconstituted in 1 mL of *n*-hexane. OCs and MX were analyzed with a Hewlett-Packard gas chromatograph (GC, HP 6890 series) equipped with a micro electron capture detector (μ-ECD). An aliquot of 1 μL was injected in splitless mode at an injector temperature of 250°C. Separation was performed on a HP-5MS capillary column (30 m x 0.25 mm) coated with 0.25 μm 5% phenyl methyl siloxane. The temperature was programmed to increase from 70°C (1 min hold) to 200°C at 20°C/min, to 230°C at 3°C/min, to 320°C at 4°C/min (10 min hold). The helium carrier gas flow rate was 1.2 mL/min, the nitrogen make-up gas flow rate was 5 mL/min. The substances were identified by means of the retention times and, additionally, by means of standard addition. Limits of detection were set at a signal over noise ratio of 2. Recoveries were determined by comparing fish extracts spiked prior with extracts spiked after extraction. Quantification of the substances was done with help of their relative response to the one of the internal standard. The limits of quantification expressed in wet weight were dependent on the lipid content of fish tissue (Table 1).

Table 1. Characteristics of compounds analyzed.

compound commonly used abbreviation and/or full name		**nature**	**RT** min	**ratio of signal to ISTD**	**LoD** pg/μL	**LoQ** (ng/g **lipid** ww[e], ww[o])	**reco-very** %
lindane	γ-isomer of hexachlorohexane	Insecticide.	9.9	1.66	0.025	**5**, 1.25, 0.05	89%
DDE	*p,p'*-dichloro-diphenyl dichloroethylene	Metabolite of DDT, insecticide.	15.3	0.74	0.025	**10**, 2.5, 0.1	76%
MX	musk xylene	Fragrance in soap and washing powder.	10.8	1.66	0.01	**20**, 5, 0.2	60%
PCB 101	2,4,5,2',5'-pentachlorobiphenyl	Indicator	14.3	0.70	0.05	**10**, 2.5, 0.1	85%
PCB 118	2,3',4,4',5'-pentachlorobiphenyl	congeners	16.6	0.54	0.2	**30**, 7.5, 0.3	94%
PCB 153	2,4,5,2',4',5'-hexachlorobiphenyl	of PCB-	17.4	0.69	0.025	**10**, 2.5, 0.1	78%
PCB 138	2,3,4,2',4',5'-hexachlorobiphenyl	mixtures	18.6	0.64	0.05	**10**, 2.5, 0.1	68%
PCB 180	2,3,4,5,2',4',5'-heptachlorobiphyl	(industrial chemicals).	21.3	0.80	0.05	**10**, 2.5, 0.1	79%
PCB 209	decachlorobiphenyl	ISTD	28.0	1	0.025	**10**, 2.5, 0.1	93%

RT: retention time, ISTD: internal standard, LoD: limit of detection, LoQ: limit of quantification (in ng/g lipids, lip, bold, and ng/g wet weight) calculated for eel (ww[e]) with 25% and for other fish (ww[o]) with e.g. 1% fat content.

RESULTS AND DISCUSSION

Persistent lipophilic compounds were measured in all fish at all sites of the Grand Duchy. Once again, the ubiquity of these contaminants in the aquatic environment was demonstrated. Lowest PCB concentrations were found in perches from the Sûre reservoir (Sûre2), highest in eels from the Moselle (Table 2). The high contamination of the Moselle is due to industry upstream in France (ancient mining watershed, CIPMS 1994b). Even though the emitting factories have been redeveloped in the meantime, the PCB concentrations in fish remain high. Eels and perches originating from a flooded quarry close to the Moselle at Remerschen (Fig. 1) had lower PCB contents (1,940 and 3,360 ng/g lipids, respectively, Vescei-Hohl 1992) than the respective species from the Moselle in this study (22,507 and 67,121 ng/g lipids respectively, Table 2). Water borne lipophilic contaminants are likely removed by the bank and are diluted with clean groundwater during the infiltration of Moselle water into the quarry. Contradictory to the high PCB fish body burdens, the water quality of the Moselle and the lower Sûre (Sûre5) is very good when using classical chemical parameters (Ministère de l'Environnement 1998). Once persistent OCs are in the water, they are, if at all, 'eliminated' only by sedimentation. As their biodegradation is low their presence is not traced out when classic chemical parameters such as the oxygen demand is measured.

Table 2. OCs and MX in perches (number of animals analyzed in brackets) and eels in ng/g weight (**bold**: ng/g lipids) in the Sûre reservoir (Sûre2) and in the Moselle.

	Sûre2, reservoir		**Moselle**	
	perch (3)	**eel (3)**	**perch (2)**	**eel (3)**
	avg. ± SD	avg. ± SD	avg.	avg. ± SD
size [cm]	20.67 ± 3.55	57.3 ± 11.02	19.0	64.7 ± 0.58
weight [g]	94.00 ± 47.79	349.0 ± 164.9	112.8	579.3 ± 72.8
lipid content [%]	0.24 ± 0.03	21.5 ± 0.56	0.7	22.3 ± 9.87
Compound				
PCB 118	0.10 ± 0.01	5.7 ± 4.3	17.47	179.4 ± 63.2
PCB 138	0.55 ± 0.17	29.0 ± 19.6	72.13	694.6 ± 209.2
PCB 153	0.54 ± 0.12	39.4 ± 24.0	88.21	783.9 ± 285.3
PCB 180	0.13 ± 0.06	16.8 ± 11.8	71.44	335.7 ± 120.6
*total PCB**	*3.27 / **1375***	*205 / **961***	*481 / **67121***	*4436 / **22507***
Lindane	1.29 ± 0.40	53.1 ± 35.4	1.16	37.4 ± 13.5
DDE	0.51 ± 0.16	42.8 ± 28.5	15.95	187.9 ± 48.2
Musk xylene	0.38 ± 0.38	12.4 ± 4.1	0.91	20.5 ± 15.7

* calculated as 3 times the sum of PCB 138 and 153.

In Tables 2 and 3 the total PCB content was calculated as 3 times the sum of PCBs 138 and 153. In biologic tissues these congeners are especially accumulated, in human tissue to a larger extent than in fish (DFG 1988, Mes et al. 1989, Zimmermann et al. 1997). The formula used to calculate total PCB content in human lipids (sum of PCB 138, 153, and 180 multiplied by 1.7) would therefore underestimate the total PCB content in fish. Extrapolation of measured PCBs 138 and 153 in relation to their presence in commercial mixtures (e.g. multiplication of measured PCB 153 by 10 because Clophen A60 contents 10% of PCB 153) would overestimate the total PCB content. In (marine) fishes from other geographic regions with different congener specific pollution, again a different extrapolation than the one used in this study may be necessary to estimate the total PCB content (Ahlborg et al. 1992a, DFG 1988, Mes et al. 1989).

Spawning allows a female fish to loose a substantial amount of its PCB body burden (Vodicnik and Peterson 1985). The few fish identified as female in this study had not lower OC concentrations than other fish, though (Table 3). It is very likely that in nature a female fish remaining in the same environment while maturing of the eggs will compensate the sequestered and spawned lipophilic compounds rather quickly. It has been shown that PCBs are uniformly distributed in the lipid compartment of the organism within 8 days (Zimmermann 1996). This explains also the finding that concentrations seem independent of the analyzed tissue (muscle, skin, eggs of bleak and gudgeon) and of the age class of the bleaks when expressed per lipid content (Table 3).

Table 3. OC and MX concentrations (in ng/g wet weight and in ng/g lipids, bold values) in bleak (*Alburnus alburnus*) and gudgeon (*Gobio gobio*). Comparison between different tissues and between adult and juvenile fish.

site	Sûre2 (reservoir)		Sûre2 (reservoir)					Our2 (Hoesdorf)			
	bleak (avg. values)		bleak No.1			bleak No.2		gudgeon No.1		gudgeon No.2	
	adult (n=3)	juv. (n=3)									
size [cm]	18.3	7	20.0			17.5		14.0		13.0	
weight [g]	50	6.2	65			45		30.8		23.3	
sex, if determinable			female					female			
nature of tissue	**muscle**	**muscle**	**skin**	**eggs**	**m'cle**	**skin**	**m'cle**	**skin**	**m'cle**	**skin**	**m'cle**
lipid content %	4.24	1.1	5.97	2.31	0.51	10.11	10.18	1.19	0.37	3.08	0.73
Compound											
total PCB*	24.8	11.9	58.7	23.0	4.8	37.5	49.0	76.6	20.2	76.5	18.9
Lindane	27.8	8.9	41.2	11.5	3.4	72.5	70.6	1.49	0.46	2.38	0.57
DDE	13.5	5.2	18.9	5.5	0.9	19.5	17.6	5.8	1.9	5.2	1.2
tot PCB	**813**	**1002**	**982**	**994**	**935**	**371**	**480**	**6434**	**5459**	**2483**	**2589**
Lindane	**606**	**726**	**690**	**498**	**657**	**717**	**692**	**125**	**123**	**77**	**79**
DDE	**170**	**272**	**316**	**237**	**173**	**193**	**172**	**276**	**263**	**170**	**159**

* calculated as 3 times the sum of PCB 138 and 153.

Fish fed to the otter related minks must not exceed PCB concentrations of 50 ng PCB/g fresh weight to ensure successful reproduction (Jensen et al. 1977). Perch from the just slightly contaminated Sûre reservoir had an average total PCB concentration of 3 ng/g wet weight, the respective concentration in eel was 205 ng PCB/g (Table 2). This can be read as that the otter could survive on a perch but not on an eel diet. Considering the 3.5 times higher energy content of eel tissue, however (1,186 kJ/100 g, Senser and Scherz 1987), eel is - expressed per energy units - only 20 times more polluted than perch (343 kJ/100 g) and can therefore still be appropriate for the otter in a mixed diet with other species. The presence of the otter has been confirmed by traces in snow found in the northwestern part of Luxembourg (Schmidt 1997).

The differences in the distribution patterns of PCBs, pesticides and MX (Fig. 2) indicate an independent contamination with industrial chemicals and/or pesticides and domestic sewage. The DDE pollution found in the Ernz noire must originate from former agricultural applications and must be 'home-made' as the Ernz noire lies entirely on Luxembourgian territory (Fig. 1 and 2). Even though DDT is nowadays not used in Western Europe any more, the distribution pattern of DDE (metabolite of DDT) is similar to the one of lindane, which is now as ever an important insecticide (Lindafor FLO®, Barenbrug, 1998). The high lindane concentrations (relative to the total contamination) in the Sûre reservoir (Sûre2, Fig. 2) can be explained by the circumstance that 26% of the reservoir catchment area is agriculturally used (compared to 14% in the whole country, L. Pfister, personal communication). Why MX has a distribution pattern similar to the two insecticides remains to be understood.

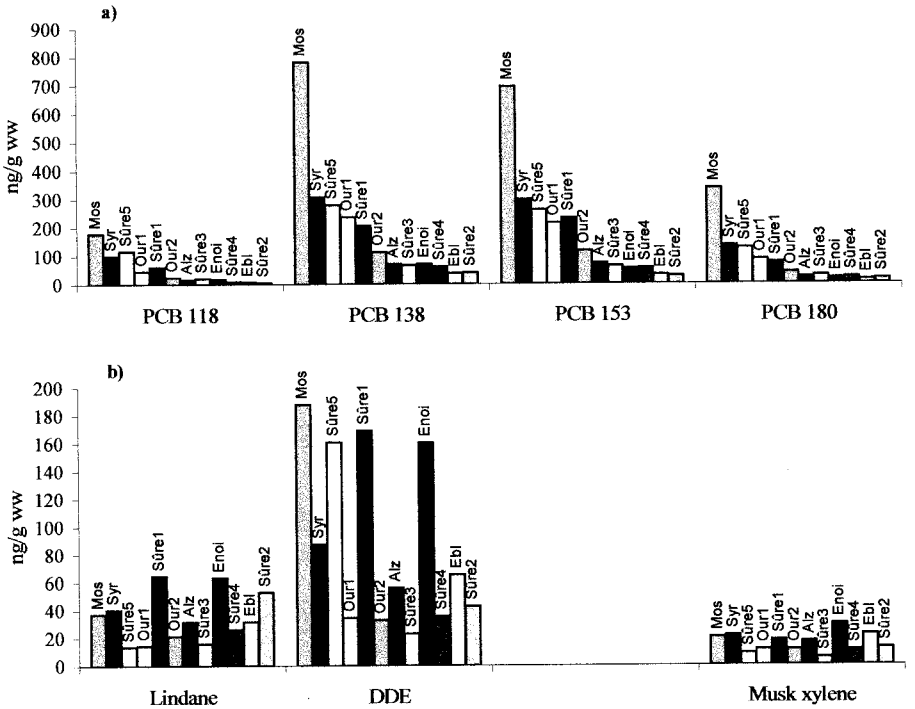

Figure 2. Distribution patterns within Luxembourg of the contaminants analyzed in eels (in ng/g wet weight, ww). Average concentrations of 3 individuals are given, except at Syr, Our1, Alz and Sûre4 where only one eel was captured and analyzed (Moselle, Alz: Alzette, Enoi: Ernz noire, Ebl: Ernz blanche, see also Fig. 1).

In contrast to mink for which a real no-observed-effect concentration exists, such a value is not known for humans. The German legislation sets a limit of 300 ng PCB 153 per g wet weight in fish designated for human consumption (SHmV 1988). Eels from the Moselle exceeded this limit by a factor of 2. It is suspected, however, that this limit is derived from known concentrations in the environment after the criterion 'as low as reasonably achievable' (Greim 2000). Setting a toxicologically justified PCB limit for humans is complicated by the fact that commercial PCBs are mixtures of some of the 209 possible PCB congeners. The acute toxicities of these mixtures, mainly composed of di-ortho substituted PCB congeners, are low (LD_{50} between 4 and 11 g/kg in rats, WHO 1993). The non- and mono-ortho substituted PCB congeners, however, have toxicities which have been compared to the one of dioxins (Safe 1990). When the concentrations of the toxic PCB congeners in food are known, the toxicity can be estimated by means of calculating the (tetrachloro-dibenzo-) dioxin equivalency (TEq). Eels from the Moselle had an average PCB 118 content of 931 ng/g lipid. PCB 118 is the most represented mono-substituted PCB in biological samples and has a toxic equivalency factor of 0.0001 (Van den Berg et al. 1998). The resulting TEq of PCB 118 in eel is 100 pg/g lipids (931,000 pg/g x 0.0001). This is 20 times above the actual limit set for PCBs in meat (expressed in TEq) after the dioxin scandal in Belgium (BgVV 2000). A meal of 100 g eel (i.e. 25 g lipids) would have a TEq of 2,500 pg, exceeding also the tolerated daily dioxin intake (1 to 4 pg/kg body weight, i.e. 70 to 280 pg for adults, WHO 1998). In these calculations the additional TEq contribution of the non-ortho substituted PCB congeners, HCB, dioxins and furans, present in fish tissue as well, has not been considered yet. If doing so, eels from the Moselle would exceed the tolerated concentrations not by a factor of 2 or 20, but rather by a factor of 200. In other words, the limit of 300 ng PCB 153/g fish would have to be lowered to 3 ng/g. Of all the fish analyzed in this study, only perch from the Sûre reservoir could meet this new standard. This would restrict the consumption of local fish to a minimum and shows interesting parallels to the discussion on the otter. Due to the high background pollution of PCBs in the aquatic environment, fish cannot meet the quality criteria set for other food sources. This can more easily be understood from the practical ('as low as reasonably achievable') than from the toxicological point of view.

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