The Handbook of Environmental Chemistry Vol. 3, Part X (2004): 151–188
DOI 10.1007/b14120

Synthetic Musks in Bioindicators: Monitoring Data of Fish and Human Milk Samples from the Czech Republic

Jana Hajšlová · Lucie Šetková

Institute of Chemical Technology, Technická 3, Department of Food Chemistry and Analysis, 16628 Prague 6, Czech Republic
E-mail: jana.hajslova@vscht.cz

Abstract Until the middle of the 1990s, no attention was paid to the pollution of the environment in Czech Republic by synthetic musk fragrances and to the exposure of Czech population to these xenobiotics. However, in response to increasing information on high levels of synthetic musks in freshwater ecosystem reported by many industrial countries in the last decade, extensive monitoring study was conducted in Czech Republic in the years 1997–2000. More than 800 samples were collected at 11 sampling sites and examined for the presence of both polycyclic and nitro musk compounds. Their occurrence in various fish employed as bioindicator of river pollution as well as investigation of human milk documented omnipresent character of these widely used synthetic chemicals. High levels of synthetic musks were typically found downstream large urban areas. The relative concentrations in almost all analysed fish samples (perch, bream, chub, barbel and trout) decreased in following order: HHCB>AHTN≫MX ≥MK≈AHDI>ATII≈ADBI. Polycyclic musks represented mainly by HHCB and AHTN accounted typically for more than 80% of total musk content; nevertheless, the bioaccumulation phenomena are distinctly species-dependent. Besides fish, application of semipermeable membrane device (SPMD) was shown to be a conceivable tool for the integral monitoring of the presence of musk containing sewage water in aquatic ecosystems. As fish represents a minor component of the Czech food basket, general occurrence of both groups of synthetic musk compounds in milk samples collected from 59 nursing mothers documented the significance of non-dietary routes of human exposure. The levels as well as relative abundance of synthetic musks varied in a wide range with polycyclic compounds prevailing in most of samples. Critical assessment of our results together with their comparison with data generated abroad in similar studies is presented in this chapter.

Keywords Fish · Human milk · Bioindicators · Polycyclic musks · Nitro musks · Aquatic ecosystem · River Elbe · River Moldau · River Tichá orlice

1 Introduction

Findings of the occurrence of synthetic musk fragrances in biota from German rivers reported by several authors [1–3] in the first part of the last decade caused in 1996 the establishment of a pilot study concerned with the examination of fish collected from the river Elbe at Hřensko (locality on the Czech-German border) for the presence of these new contaminants. As anticipated, the ubiquitous character of synthetic musk compounds was confirmed. Relatively extensive contamination of examined fish samples was documented; both representatives of polycyclic musk fragrances and nitro musks were found. With regard to the growing concern about aquatic ecosystem pollution, these compounds have involved since 1997 with the Czech monitoring program (established by the Ministry of Environment in 1994). Passive biological monitoring (PBM) approach utilizing fish for this purpose has been applied.

Musk compounds were also analysed in a set of human milk samples which were collected within another project focusing on the occurrence of persistent organic pollutants in this kind of bioindicator. Results obtained within both these studies are reported and discussed in this chapter.

2 Fish as Bioindicator: Set-up of Monitoring Study

In accordance with the common practice [4] used for the monitoring of persistent organochlorine micro-contaminants in freshwater and marine ecosystems, fish served as the main bioindicator of river pollution. Within four monitoring years (1997–2000) over 800 freshwater fish samples were collected using electro fishing at 11 sampling sites located along river Elbe and its tributaries, the rivers Moldau and Tichá Orlice in Czech Republic (Fig. 1); a few samples were also collected in the locality Podolí, upstream of Prague (very old specimen or those of extreme weight within the particular set of fish species were excluded).

Since eel, which has been widely used as a very suitable bioindicator organism of water pollution [4], did not occur in sufficient quantities in these localities, we employed several other species representing different categories as regards the typical feeding habits, wandering behaviour etc. for this purpose. Chub (*Leuciscus cephalus*), bream (*Abramis brama*), barbel (*Barbus barbus*) and perch (*Perca fluviatilis*) collected from rivers Elbe (Labe) and Moldau (Vltava) represent abundant fish species available in all localities each year. In the river Tichá Orlice, trout (*Salmo trutta*) is the only available fish. It should be emphasized that the migration of fish along the rivers was very restricted because of dams and/or weirs surrounding sampling localities. Sampling of fish was carried out regularly in all localities in the period between July and September by the University of Southern Bohemia, Research Institute of Fish Culture and Hydrobiology (Vodňany, Czech Republic).

Frozen samples (fillets, skin removed) that were delivered to our laboratory were held at –18 °C until analysis. The sets of examined fish from individual rivers are characterized in detail (Tables 1, 2 and 3).

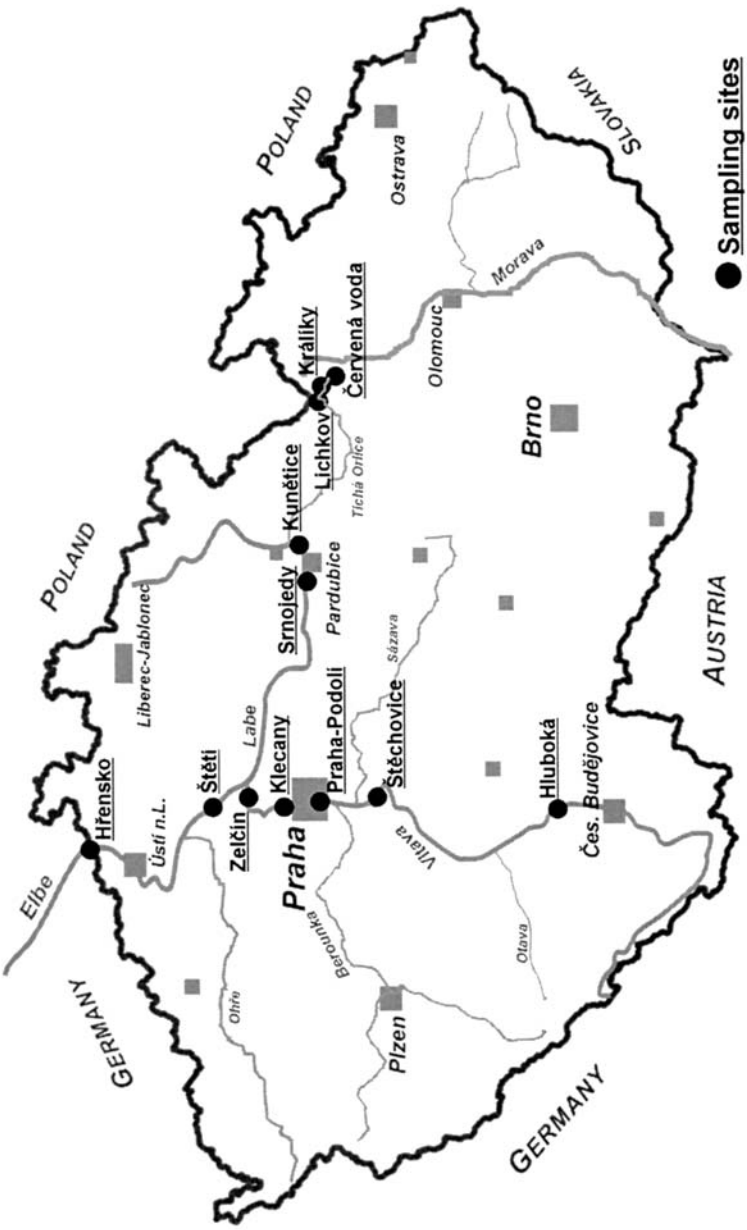

Fig. 1 Sampling sites at rivers Elbe (Labe), Moldau (Vltava) and Tichá Orlice

Table 1 Characteristics of fish samples from river Elbe (Labe) examined within monitoring program

Fish species	Characteristics of samples collected during monitoring program in particular localities (1 – Kunětice, 2 – Srnojedy, 3 – Štětí, 4 – Hřensko)															
	1997				1998				1999				2000			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Chub																
No. of pooled/ individual samples	2 (6)[a]	1 (5)	4 (17)		3 (10)	3 (10)	3 (10)	3 (11)	4 (20)	4 (17)	4 (20)	4	11	10	10	27
Mean length (mm)	320	304	284		334	300	385	383	394	342	445	443	289	274	368	210
RSD (%)	12	12	9		7	14	8	8	8	13	7	7	13	20	19	58
Mean fat (wt%)	1.1	1.5	1.8		1.0	1.0	1.4	2.2	2.2	1.6	1.5	3.0	1.3	2.5	1.8	4.0
RSD (%)	9		33		20	40	7	14	45	19	20	33	38	68	44	28
Bream																
No. of pooled/ individual samples	1 (4)[a]	2 (8)	1 (2)	20	2 (5)	3	3 (4)	3 (10)	3 (7)	4 (15)		21	5		10	6
Mean length (mm)	201	248	252	302	335	412	212	336	392	482		401	282		266	326
RSD (%)	8	27	4	10	14	4	16	12	4	7		11	11		17	16
Mean fat (wt%)	0.7	0.8	1.8	1.7	0.7	6.4	1.6	1.9	1.1	5.0		2.9	1.2		1.9	0.7
RSD (%)		13		41	14	30	6	25	16	14		10	33		26	43

Table 1 (continued)

Fish species	Characteristics of samples collected during monitoring program in particular localities (1 – Kunětice, 2 – Srnojedy, 3 – Štětí, 4 – Hřensko)															
	1997				1998				1999				2000			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Barbel																
No. of pooled/ individual samples	1	1	1	1		3			2 (3)	3		3	8			3
Mean length (mm)	468	472	455	460		353			347	408		424	433			456
RSD (%)						1			31	8		13	6			17
Mean fat (wt%)	2.6	4.9	7.3	0.6		3.2			2.5	2.4		9.2	2.7			6.5
RSD (%)						22			36	8		15	44			32
Perch																
No. of pooled/ individual samples	1 (2)[a]	1 (5)		1			3		2 (11)	3 (15)	3 (8)		10		5	6
Mean length (mm)	163	185		205			146		148	216	188		155		161	154
RSD (%)	4	12					24		24	6	8		10		12	21
Mean fat (wt%)	0.6	0.7		0.6			0.6		0.7	0.6	0.6		0.7		0.8	0.9
RSD (%)							17		14	17	14		29		13	11

[a] In brackets: total number of fish samples composing pool samples.

Table 2 Characteristics of fish samples from river Moldau (Vltava) examined within monitoring program

Fish species	Characteristics of samples collected during monitoring program in particular localities (1 – Hluboká, 2 – Štěchovice, 3 – Klecany, 4 – Zelčín)											
	1998				1999				2000			
	1	2	3	4	1	2	3	4	1	2	3	4
Chub												
No. of pooled/ individual samples	8 (18)[a]		6 (17)		3 (10)	2 (3)	18		10	9	20	9
Mean length (mm)	258		258		296	288	300		322	316	323	300
RSD (wt%)	20		22		18	12	11		21	18	17	18
Mean fat (%)	0.8		2.0		2.7	2.7	4.2		2.6	2.5	3.0	2.2
RSD (%)	25		5		56	30	48		35	32	30	60
Bream												
No. of pooled/ individual samples	5 (12)[a]		3		2 (3)		2				14	10
Mean length (mm)	244		419		298		488				362	308
RSD (%)	13		7		3		6				7	8
Mean fat (wt%)	1.2		5.1		1.6		6.7				4.2	0.7
RSD (%)	8		16		13		24				93	43

Table 2 (continued)

Fish species	Characteristics of samples collected during monitoring program in particular localities (1 – Hluboká, 2 – Štěchovice, 3 – Klecany, 4 – Zelčín)											
	1998				1999				2000			
	1	2	3	4	1	2	3	4	1	2	3	4
Barbel												
No. of pooled/ individual samples			3 (10)[a]				3 (10)				3	
Mean length (mm)			408				471				408	
RSD (%)			10				10				11	
Mean fat (wt%)			3.2				5.3				4.3	
RSD (%)			19				19				5	
Perch												
No. of pooled/ individual samples		5 (14)	4 (11)[a]			3 (9)	18		7	13	18	
Mean length (mm)		162	166			214			204	185	227	
RSD (%)		26	17			6			39	21	25	
Mean fat (wt%)		0.3	0.7			0.7			0.9	0.8	0.9	
RSD (%)		33	14			14			11	25	33	

[a] In brackets: total number of fish samples composing pool samples.

Table 3 Characteristics of fish samples from river Tichá Orlice examined within monitoring program

Fish species	Characteristics of samples collected during monitoring program in particular localities (1 – Červená voda, 2 – Králíky, 3 – Lichkov)								
	1998			1999			2000		
	1	2	3	1	2	3	1	2	3
Trout									
No. of pooled/ individual samples	3 (13)[a]	3 (10)	3 (14)	3 (15)	3 (15)	3 (15)	3 (12)	3 (12)	3 (11)
Mean length (mm)	173	224	221	197	267	250	160	208	202
RSD (%)	12	13	12	11	9	8	11	7	10
Mean fat (wt%)	0.6	1.7	1.0	0.8	2.5	0.9	1.8	2.4	2.3
RSD (%)	33	18	59	13	12	22	6	13	9

[a] In brackets: total number of fish samples composing pool samples.

With the exception of the year 2000, in which each fish was analysed individually, pooling of samples was carried out to rationalize the number of analyses. To avoid the loss of information needed for an assessment of bioaccumulation, pooling strategy was based on the assumption that the length of fish is typically proportional to its age (which corresponds to the duration of the residence of fish in contaminated water). Samples from each locality were sorted into several groups according to their relative length. This parameter (alike the weight) was shown to correlate well with the relative age of the specimen within the population of collected samples. The lipid content extracted under conditions of analytical procedure applied for musk analysis was recorded for each analytical sample. As can be seen from Tables 1, 2 and 3, the among-season variation of both the mean lengths and lipid contents of fish caught in individual localities was relatively large, reflecting not only some variance due to the sampling process itself but also the influence of conditions existing in respective aquatic ecosystem, in particular sampling year (the later factor significantly determines the form of resident biota). The highest variability was found in the bream population. For instance the relative standard deviation (RSD) calculated for lengths of the whole set of these species collected along the river Elbe through the year 2000 was 25%, for the chub population having approximately the same mean length this value was about 18%, for examined barbel the RSD of lengths was the lowest (10%). A perspicuous correlation between the mean length of particular fish species and their lipid contents even in a single sampling period has not been found in any of the sampling localities. The highest correlation coefficient (0.8) was calculated for chub (n=20) and perch (n=18) from Klecany in the sampling year 2000.

Although fish was used as a main bioindicator of pollution situation at all localities (as well as fish fillets, some samples of livers were also examined for this

purpose), samples of sediments were also collected here. Wet material was sieved, carefully dried and then stored in a refrigerator.

To obtain complementary information on the occurrence of synthetic musks in aquatic ecosystem and to receive complementary data needed for the assessment of bioaccumulation/metabolisation processes in fish, passive sampling utilizing semipermeable membrane devices (SPMDs) was carried out in several localities.

Regarding the target analytes, HHCB and AHTN representing polycyclic musk fragrances together with two major nitro musks – musk ketone (MK) and musk xylene (MX) – were determined in all samples throughout the whole monitoring program. In the last two years, an additional three polycyclic musks with lower production volumes and usage such as ADBI, AHDI, and ATII were analysed in fish from all localities.

Multiresidue analytical procedure used for all monitored musks was similar to that described by Rimkus et al. [5]. It consisted of following steps: (i) Soxhlet extraction of homogenized fish tissue (anhydrous sodium sulphate was added for dehydration) by hexane-dichloromethane solvent mixture (1:1, v/v), (ii) removal of bulk lipids from crude extract by automated gel permeation chromatography (GPC; Gilson 231XL, France) using Bio-Beads S-X3 gel (Bio-Rad Laboratories, USA) and cyclohexane-ethyl acetate (1:1, v/v) as a mobile phase, (iii) removal of residual matrix co-extracts by adsorption chromatography on silica gel mini-column (elution of analytes by 15% of diethyl ether in hexane, v/v), (iv) identification/quantitation of analytes by GC/MS (GC-HP 6890; DB-5MS capillary column, J&W Scientific, USA; MSD-HP 5973, Agilent Technologies, USA) operated in EI mode. The performance characteristics of this method (SIM, three characteristic ions monitored) are summarized in Table 4.

For gravimetric determination of lipids, aliquot portion of extract obtained in step (i) was used.

2.1 Synthetic Musks in Fish Samples from Selected Sites at Czech Rivers

The overview of synthetic musks levels analysed in fish samples throughout the monitoring program is given in Tables 5, 6 and 7 (results of 461 analyses are

Table 4 Performance characteristics of the GC/MS (SIM) method used for synthetic musk analysis of fish samples (isotope dilution technique using labeled D_3-AHTN and D_{15}-musk xylene as internal standards for quantitation)

Analytes	Limit of detection (LOD, µg kg^{-1} lw)	RSD (%) (at 10 LOD level, n=6)	Recovery (%) (at 10 LOD level)
HHCB (e.g. Galaxolide)	3	7	93
AHTN (e.g. Tonalide)	3	8	85
ADBI (e.g. Celestolide)	1	10	87
AHDI (e.g. Phantolide)	1	9	86
ATII (e.g. Traseolide)	1	11	85
MX (musk xylene)	5	11	82
MK (musk ketone)	5	9	91

Table 5 Musk compounds in fish from river Elbe (Labe), monitoring data 1997–2000

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)															
		Kunětice				Srnojedy				Štětí				Hřensko			
		1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000
Chub	HHCB																
	Mean	695	709	631	1088	707	1169	1405	1146	840	1402	1049	849		672	739	932
	RSD (%)	14	24	10	17		26	8	21	51	18	27	23		13	26	35
	Median	695	736	632	1145		1233	1420	1175	673	1402	1059	886		708	703	791
	AHTN																
	Mean	655	735	647	957	739	950	1330	1052	888	1219	702	695		957	677	765
	RSD (%)	8	32	27	19		30	10	19	46	2	24	21		23	21	27
	Median	655	824	595	996		1152	1303	1096	740	1219	764	735		963	650	723
	MX																
	Mean	172	92	53	151	221	141	120	97	238	191	77	49		544	157	239
	RSD (%)	11	11	42	25		26	18	31	42	9	22	12		25	18	30
	Median	172	93	55	166		156	125	92	251	191	83	47		470	169	219
	MK																
	Mean	144	125	56	124	222	104	78	54	120	120	57	55		118	52	76
	RSD (%)	10	26	23	23		16	14	37	48	8	14	31		11	12	25
	Median	144	126	54	144		115	78	59	100	120	56	56		110	49	71
	ADBI																
	Mean		28	91			41	34			25	25			18	19	
	RSD (%)		30	32			32	68			16	36			17	63	
	Median		25	107			35	26			26	22			17	19	

Table 5 (continued)

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)															
		Kunětice				Srnojedy				Štětí				Hřensko			
		1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000
	AHDI																
	Mean		54	221			92	60			50	56			40	49	
	RSD (%)		31	23			20	35			22	38			20	31	
	Median		48	255			86	57			52	48			36	45	
	ATII																
	Mean		58	108			95	36			25	32			20	28	
	RSD (%)		48	17			32	39			28	34			20	39	
	Median		57	116			106	34			25	30			19	29	
Bream	HHCB																
	Mean	915	3552	2001	1915	1904	2817	6364		492	4510		5417	1943	3655	2324	2816
	RSD (%)		9	6	18	13	11	5			16		27	24	15	39	25
	Median		3552	2051	1975	1904	2848	6444			4510		5526	1943	3448	2398	2687
	AHTN																
	Mean	585	1806	1210	1479	917	1162	2165		582	1194		1374	975	2135	1144	1359
	RSD (%)		3	11	24	18	13	13			57		51	19	14	25	30
	Median		1806	1260	1385	917	1083	2195			1194		1018	987	2012	1116	1305
	MX																
	Mean	149	159	99	140	279	147	86		68	304		81	129	610	230	169
	RSD (%)		10	12	30	13	7	33			25		36	91	39	20	27
	Median		159	103	129	279	143	87			304		75	105	514	232	153

Table 5 (continued)

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)															
		Kunětice				Srnojedy				Štětí				Hřensko			
		1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000
	MK																
	Mean	167	149	97	96	191	95	110		167	118		69	338	129	56	72
	RSD (%)		9	14	10	1	11	12			1		41	28	17	21	19
	Median		149	98	99	191	90	106			118		70	323	121	56	70
	ADBI																
	Mean			48	25			49					34			37	34
	RSD (%)			8	12			18					50			19	41
	Median			49	23			48					24			39	31
	AHDI																
	Mean			123	78			174					84			69	85
	RSD (%)			7	22			22					44			16	33
	Median			125	82			189					78			69	78
	ATII																
	Mean			110	35			103					59			42	46
	RSD (%)			15	37			17					73			19	41
	Median			111	35			106					49			47	38
Barbel	HHCB																
	Mean	1871		2250	2373	3731	3207	3933		342				1127		2263	3792
	RSD (%)			12	8		17	12								20	
	Median			2250	2304		3279	4176								2587	

Table 5 (continued)

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)															
		Kunětice				Srnojedy				Štětí				Hřensko			
		1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000
	AHTN																
	Mean	1743		1319	2209	985	2715	2722		465				1206		1715	1653
	RSD (%)			32	11		15	19								18	
	Median			1319	2253		2862	3004								1908	
	MX																
	Mean	230		61	131	215	324	135		51				87	368	258	
	RSD (%)			5	11		17	18							13		
	Median			61	1239		323	125							389		
	MK																
	Mean	103		53	64	192	122	67		28				385	62	82	
	RSD (%)			4	8		11	3							10		
	Median			53	63		126	66							65		
	ADBI																
	Mean			17	14			26							17	18	
	RSD (%)			18	21			15							6		
	Median			17	15			24							18		
	AHDI																
	Mean	136	140					192							122	113	
	RSD (%)	22	11					48							14		
	Median	136	136					204							131		
	ATII																
	Mean	47	37					150							37	33	
	RSD (%)	21	5					58							11		
	Median	47	38					110							40		

Table 5 (continued)

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)															
		Kunětice				Srnojedy				Štětí				Hřensko			
		1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000
Perch	HHCB																
	Mean	896		1456	1123	2437		2853			1954	5318	392				956
	RSD (%)			26	20			23			29	33					12
	Median			1294	1048			2990			2035	5847					956
	AHTN																
	Mean	980		1289	1055	967		2648			1390	1239	911				797
	RSD (%)			29	19			35			27	41					1
	Median			1133	1009			2763			1622	1025					797
	MX																
	Mean	88		109	189	195		173			162	117	33				235
	RSD (%)			4	30			15			5	55					14
	Median			107	186			190			167	107					235
	MK																
	Mean	86		96	134	296		155			129	127	170				128
	RSD (%)			6	19			18			12	68					24
	Median			92	130			165			121	103					128
	ADBI																
	Mean		68	86			91	99	67								36
	RSD (%)		10	35			18	25	63								3
	Median		70	102			101	89	70								36

Table 5 (continued)

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)															
		Kunětice				Srnojedy				Štětí				Hřensko			
		1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000
	AHDI																
	Mean		141	207			242		173	153							81
	RSD (%)		21	31			21		6	63							17
	Median		151	236			262		171	156							81
	ATII																
	Mean		161	126			288		148	115							37
	RSD (%)		14	42			24		36	83							35
	Median		149	127			287		118	98							37

Table 6 Musk compounds in fish from river Moldau (Vltava), monitoring data 1998–2000

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)											
		Hluboká nad Vltavou			Štěchovice			Klecany			Zelčín		
		1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000
Chub	HHCB												
	Mean	971	1351	821		368	707	2693	1274	1555			855
	RSD (%)	29	35	22		30	28	25	23	24			22
	Median	904	1607	793		368	704	2724	1240	1535			804
	AHTN												
	Mean	1019	753	520		232	407	2448	831	1283			802
	RSD (%)	34	33	25		24	22	19	18	29			21
	Median	878	794	535		232	445	2390	888	1188			759
	MX												
	Mean	160	100	65		104	32	494	160	169			146
	RSD (%)	41	40	23		22	25	14	14	26			13
	Median	146	110	58		104	33	534	160	165			136
	MK												
	Mean	121	85	37		180	24	213	121	127			87
	RSD (%)	33	32	25		16	17	14	15	18			16
	Median	107	101	39		102	25	232	103	123			86
	ADBI												
	Mean		41	61		96	16		41	22			24
	RSD (%)		24	18		29	19		22	45			83
	Median		45	63		96	18		47	21			18

Table 6 (continued)

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)											
		Hluboká nad Vltavou			Štěchovice			Klecany			Zelčín		
		1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000
	AHDI												
	Mean		79	76		94	23		72	76			51
	RSD (%)		14	20		29	48		54	25			27
	Median		75	79		94	20		77	77			52
	ATII												
	Mean		45	60		83	14		34	34			34
	RSD (%)		20	22		30	29		26	29			32
	Median		39	61		83	13		36	34			42
Bream	HHCB												
	Mean	3299	1189					8654	5052	6394			2205
	RSD (%)	14	13					7	3	114			26
	Median	3299	1156					8394	5052	7971			2146
	AHTN												
	Mean	1866	1112					4764	1784	6940			1454
	RSD (%)	6	18					10	13	102			42
	Median	1866	1023					4494	1784	3470			1375
	MX												
	Mean	133	130					759	360	829			202
	RSD (%)	3	5					26	3	115			24
	Median	133	130					828	360	431			212

Table 6 (continued)

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)											
		Hluboká nad Vltavou			Štěchovice			Klecany			Zelčín		
		1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000
	MK												
	Mean	102	130					228	123	412			119
	RSD (%)	6	6					22	10	125			28
	Median	102	130					225	123	179			115
	ADBI												
	Mean		86						68	103			29
	RSD (%)		26						16	63			79
	Median		105						68	78			25
	AHDI												
	Mean		158						253	474			121
	RSD (%)		20						12	78			16
	Median		146						253	302			122
	ATII												
	Mean		117						88	180			62
	RSD (%)		28						6	113			27
	Median		98						88	104			57
Barbel	HHCB												
	Mean							10806	4616	2972			
	RSD (%)							17	12	11			
	Median							10844	4445	2745			

Table 6 (continued)

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)											
		Hluboká nad Vltavou			Štěchovice			Klecany			Zelčín		
		1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000
	AHTN												
	Mean							12026	3049	2284			
	RSD (%)							19	27	20			
	Median							11378	2563	2143			
	MX												
	Mean							1956	524	325			
	RSD (%)							17	19	30			
	Median							1878	518	315			
	MK												
	Mean							659	180	159			
	RSD (%)							29	8	18			
	Median							584	189	143			
	ADBI												
	Mean								67	106			
	RSD (%)								10	8			
	Median								67	104			
	AHDI												
	Mean								448	233			
	RSD (%)								14	18			
	Median								412	215			

Table 6 (continued)

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)											
		Hluboká nad Vltavou			Štěchovice			Klecany			Zelčín		
		1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000
	ATII												
	Mean								107	59			
	RSD (%)								18	17			
	Median								95	56			
Perch	HHCB												
	Mean			2837	784	578	2394	5446	5753	1670			
	RSD (%)			25	54	9	43	8	81	31			
	Median			2749	509	553	2329	5482	5753	1568			
	AHTN												
	Mean			1468	706	413	1895	3547	1248	1470			
	RSD (%)			21	53	8	35	11	65	32			
	Median			1521	514	402	1782	3717	1248	1443			
	MX												
	Mean			181	254	112	189	506	362	247			
	RSD (%)			40	24	4	56	10	50	35			
	Median			156	283	110	181	520	362	232			
	MK												
	Mean			180	194	145	179	143	240	193			
	RSD (%)			59	25	8	66	13	41	53			
	Median			140	223	143	165	150	255	179			

Table 6 (continued)

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)											
		Hluboká nad Vltavou			Štěchovice			Klecany			Zelčín		
		1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000
	ADBI												
	Mean			98		69	127		284	192			
	RSD (%)			46		9	62		37	34			
	Median			67		65	128		284	178			
	AHDI												
	Mean			145		92	228		386	271			
	RSD (%)			8		17	56		48	27			
	Median			142		89	239		386	279			
	ATII												
	Mean			113		99	175		236	234			
	RSD (%)			38		49	56		48	27			
	Median			103		78	183		236	230			

Table 7 Musk compounds in fish from river Tichá Orlice, monitoring data 1998–2000

Fish species	Analyte	Content of analyte in fish from particular localities (μg kg^{-1} lw)								
		Červená Voda			Králíky			Lichkov		
		1998	1999	2000	1998	1999	2000	1998	1999	2000
Trout	HHCB									
	Mean	271	586	489	2330	1000	1787	713	1371	1628
	RSD (%)	11	63	12	16	34	7	42	61	3
	Median	266	343	475	2233	1096	1335	819	876	1606
	AHTN									
	Mean	282	399	333	3125	642	1022	1181	858	614
	RSD (%)	14	25	5	9	15	8	41	23	52
	Median	286	363	334	3156	702	970	1264	825	793
	MX									
	Mean	187	162	80	1792	224	446	294	226	384
	RSD (%)	13	20	3	8	16	9	50	31	65
	Median	181	162	79	1740	216	452	349	201	149
	MK									
	Mean	105	111	57	188	112	84	200	149	102
	RSD (%)	13	5	2	12	29	11	43	25	18
	Median	98	112	58	184	91	78	219	128	89
	ADBI									
	Mean		116	33		55	82		121	37
	RSD (%)		16	6		15	5		26	5
	Median		106	32		57	81		101	37
	AHDI									
	Mean		140	62		119	111		213	79
	RSD (%)		17	3		16	26		14	3
	Median		130	63		130	97		201	79
	ATII									
	Mean		67	40		45	50		93	40
	RSD (%)		19	3		22	6		33	3
	Median		62	40		46	49		74	41

summarized here). As documented throughout this chapter, the pertinence of information mediated by generated data is dependent on a comprehensive characterization of actual fish samples submitted for analysis as well as on the knowledge of conditions existing in a particular locality in respective sampling season (water flow, its temperature etc.).

In agreement with most of similar monitoring studies concerned with occurrence of lipophilic persistent chemicals in biota, the content of musks in examined samples was normalized to the lipid contents. However, the extent of sampling site contamination was sometimes more cogently illustrated by the expression of contaminants content in muscle/liver tissue. The total body burden of respective bioindicator can be better appraised in this way. Moreover, for the risk assessment

of the dietary intake of hazardous chemicals contained in fish, information on their concentration in edible portions (fish fillets were examined in this study) is more relevant. As mentioned earlier, large differences in mean lipid content were found in population of particular fish species along monitored rivers. The reason for uneven adiposity of fish is undoubtedly the variable availability of food; its amount depends on trophic value of the water. The presence of ample macrozoobenthos is typical for mesotrophic waters. In this context, communal sewage might be a good source of nitrogen and phosphorus needed for its development. As long as the amount of oxygen remains sufficient for biota, the presence of abundant food is reflected by the rise of fish fatness in such (contaminated) locality. Under these conditions, bioconcentration factors on a wet weight basis are expected to increase due to a higher lipid content in organisms, although this does not necessarily result in elevated concentrations of contaminants in lipid [6]. In Fig. 2 various expressions of synthetic musk concentrations in bream obtained from two sampling sites of river Elbe in the years 1997–1999 (unfortunately no bream were available in the sampling year 2000 at these localities) are shown.

While, compared to samples from Kunětice, increased levels of both musk groups in tissues of bream from Srnojedy were apparent throughout the entire monitoring period indicating the closeness of pollution source (urban area Pardubice is located upstream of this sampling site), normalization of musk levels to lipid content rather obscured (see data from years 1998 and 1999) the differences between these two localities as regards the extent of their contamination. Although higher musk levels in locality Srnojedy were also documented by analysis of bottom sediments, corresponding concentrations of musks in lipid might be levelled due to the "diluting effect" occurring in fattier fish. Rather surprisingly, contamination of bream was very low in both localities close to the city Pardubice in the first monitoring year 1997. According to our records, long-term rains in respective summer season caused unusually high water flows in particular section of river Elbe what might have caused dilution of wastewater containing musk compounds and, consequently, lowered the extent of their uptake by fish. Another factors which are probably responsible for apparently low body burden of musks in bream from Srnojedy in 1997 is their small mean size (respectively age) as well as lower content of lipids compared to fish population collected in subsequent years.

Overall, polycyclic musks HHCB and AHTN were clearly dominating compounds in all fish samples as shown in a recent survey by Rimkus [7a]. Similar findings are reported in all studies carried out in Europe. The presence of other compounds of this group (ADBI, AHDI and ATII) could be unequivocally analysed in our samples too; nevertheless, their levels were lower by at least one order of magnitude. The nitro musk fragrances MX and MK were also proved ubiquitous in all monitored aquatic ecosystems. When comparing their typical abundance with polycyclic musks, the following concentration order was found in most of fish samples: HHCB>AHTN$\gg$MX$\geq$MK$\approx$AHDI>ATII$\approx$ADBI. It is worth noting that, in contrast to our data, significantly higher residue levels of MK compared to MX were reported in several German studies [2c, 7b,c, 8] for various freshwater fish, both from rivers (including river Elbe) and sewage ponds. In their comprehensive

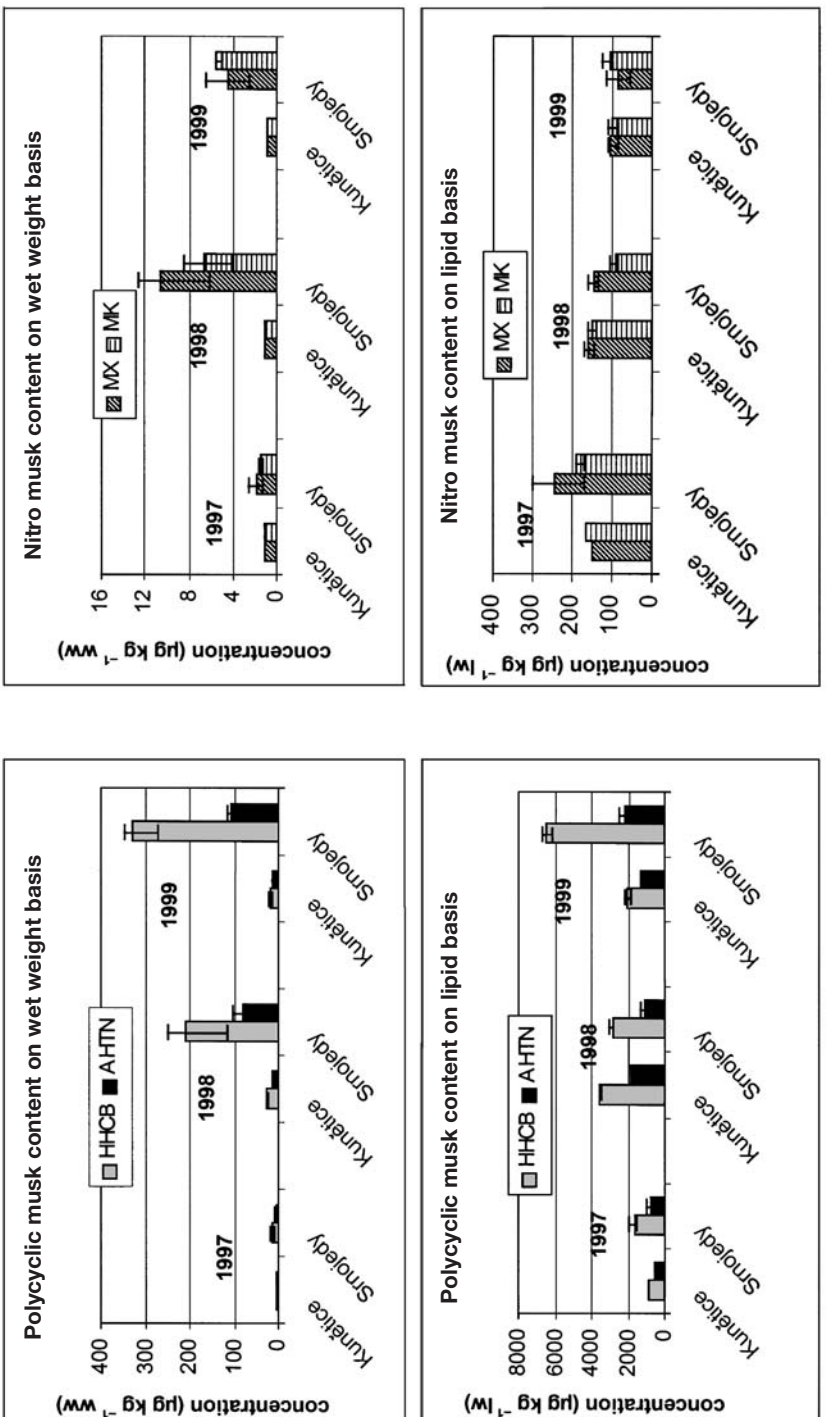

Fig. 2 Concentrations (medians) of synthetic musk compounds in breams from sampling sites located upstream (Kunětice) and downstream (Srnojedy) of urban area Pardubice

review on the fate of nitro musks in the aquatic environment, Rimkus et al. [9] referred to an important role of metabolization pathway in sewage plants resulting in a formation of monoamino metabolites from parent compounds; their presence was proved in various compartments of aquatic environment [10a,b, 11]. Adsorption in sludge is another factor influencing concentration of synthetic musks and related compounds in effluents. Although P_{ow} value (3.8) estimated for 4-NH_2-MX (main MX metabolite) is by about one order of magnitude lower than that of parent compound (P_{ow}=4.9), its hydrophobicity is still high enough to allow bioaccumulation in fish (NH_2-MK, the main metabolite of MK is too polar in this context). As shown by Rimkus et al. [9], in some fish from the German part of the river Elbe and mainly in biota from sewage ponds, 4-NH_2-MX levels exceeded its precursor even by one order of magnitude. Consequently, MX/MK ratio≤1 was determined in fish in spite of lower bioconcentration factor of MK. Concentration of nitro musks in water has to be considered for the complex assessment of bioconcentration; nevertheless, opposite situation could be expected due to the higher production rates of MX compared to MK [1a]. However, since the first findings of nitro musks in the environment and human samples at the beginning of the 1990s, the industrial production and use of MX has been significantly reduced [7c]. Rather distinct nitro musks pattern recognized in fish from Czech rivers may be a result of differences in technologies employed in sewage treatment plants; biodegradation processes leading to transformation of MX might be less extensive than those in the above-mentioned German studies. The other alternative, i.e. different pattern of use volumes of individual nitro musks in the Czech Republic compared to other countries, is not probable because practically the same cosmetic/detergent products are available in both markets. Assumption on a lower conversion rate of MX into its amino metabolite in Czech sewage treatment facilities was confirmed within a pilot study in which sets of three fish species from Klecany (Moldau, sampling year 2000, characteristics of examined set of fish is shown in Tables 1, 2 and 3 were analysed not only for MX and MK but also for their metabolites represented by 2-NH_2-MX, 4-NH_2 -MX and 2-NH_2-MK. Although 4-NH_2-MX (no other amino musks above LOD 10 μg kg^{-1} lw were detected) exceeded concentration of MX in all samples, the metabolite/parent compound concentration ratio varied among the fish species: the maximum value of 2.2 (RSD=10%) was found in perch; in chub it was 1.9 (RSD=12%) and in bream only 1.6 (RSD=10%). Either differences in bioconcentration mechanism or in the extent of metabolization of nitro musks in fish may explain these observations. In any case, in accordance with the lowest content of 4-NH_2-MX, the highest MX/MK ratio was recorded in the later species in locality Klecany – not only in the year 2000 but also in previous sampling periods.

Generally, high correlation coefficients between concentrations of the main polycyclic musks (HHCB and AHTN) existing for all examined fish species (typically r^2>0.9) were not surprising because only mixtures of these compounds are used for fragrance compositions. Regarding nitro musks, less distinct correlation between MK and MX levels in fish was calculated in some cases. Varying extent of their biotransformation discussed above might explain this fact.

Considering the whole set of data obtained for fish used as pollution bioindicator in monitored localities, the worst situation with respect to musk levels was

recognized in the localities of Klecany at Moldau, Srnojedy at Elbe and Králíky at Tichá Orlice. In all cases the sampling sites were located only a few kilometers downstream from large urban areas at which production of large volumes of sewage waters is common. Invariably, the most serious contamination of aquatic ecosystem was recognized at Klecany located approximately 4 km downstream of the outlet of one of the biggest Prague municipal sewage treatment plant. The absolutely highest concentrations (90% percentile, mg g^{-1} lw) were found in1998 in barbel: HHCB 12.6, AHTN 13.3, MX 2.3 and MK 0.9. Unfortunately, no corresponding data for this particular species (typically living in close contact with bottom sediments) are available in the literature for comparison. Nevertheless, considering the levels of polycyclic musks in bream and perch measured throughout the monitoring program in Klecany, the extent of their contamination was approximately two times higher than "the worst case situation" reported by Eschke et al. [2] in 1995 for the same species collected from the extensively polluted river Ruhr.

In accordance with findings reported, e.g. for contamination of various freshwater fish caught in several Italian rivers [12] or more recently in the river Elbe [13], HHCB levels (both, means and medians) always exceeded those of AHTN in the Czech fish samples. On the other hand, the opposite ratio for these major polycyclic musks was recorded for instance by Eschke et al. [2d], although HHCB levels in water samples taken from river Ruhr at the same time period (1994–1996) reported by the same author [2c,e] were higher compared to AHTN. It should be emphasized that environmental behaviour of these chemicals is influenced by many factors and without the knowledge of the actual pollution situation in particular compartments (levels of musks in water, suspended particulate matter and sediments) it is difficult to discuss the reason of this phenomenon.

As a part of critical assessment of the whole set of data generated throughout the monitoring program, occurrence of temporal trends in concentrations of both musk groups measured in all localities has been checked. In contrast with some reports on increasing tendency of polycyclic musks with concurrent decrease of nitro musks discussed by Rimkus [9], no statistically significant tendency of this kind was recognized in Czech rivers for either musks group. As illustrated in Fig. 3, only small variations of musk levels in specific sampling localities (less and seriously polluted ones were selected) occurred within three monitoring years both in fish tissues and sediments, in spite of some inter-annual changes in characteristics of particular matrices.

Differences in lipid contents in case of biota or slight changes in content of organic carbon in sediments may influence concentration of target pollutants. These observations may be due to a relatively insignificant fluctuation in composition of effluents from sewage treatment plants. Limited variation of musk concentrations in SPMDs deployed in river Elbe [15] as well as in various surface water samples [16] has been recently reported by other authors.

Another example of three years monitoring data obtained in our project is shown in Fig. 4 for trout collected at three localities along the small river Tichá Orlice.

As documented here, relatively low levels of musks were consistently measured in fish from sampling site downstream of the small village Červená Voda (low lipid contents in local fish were due to a limited availability of food in this "clean"

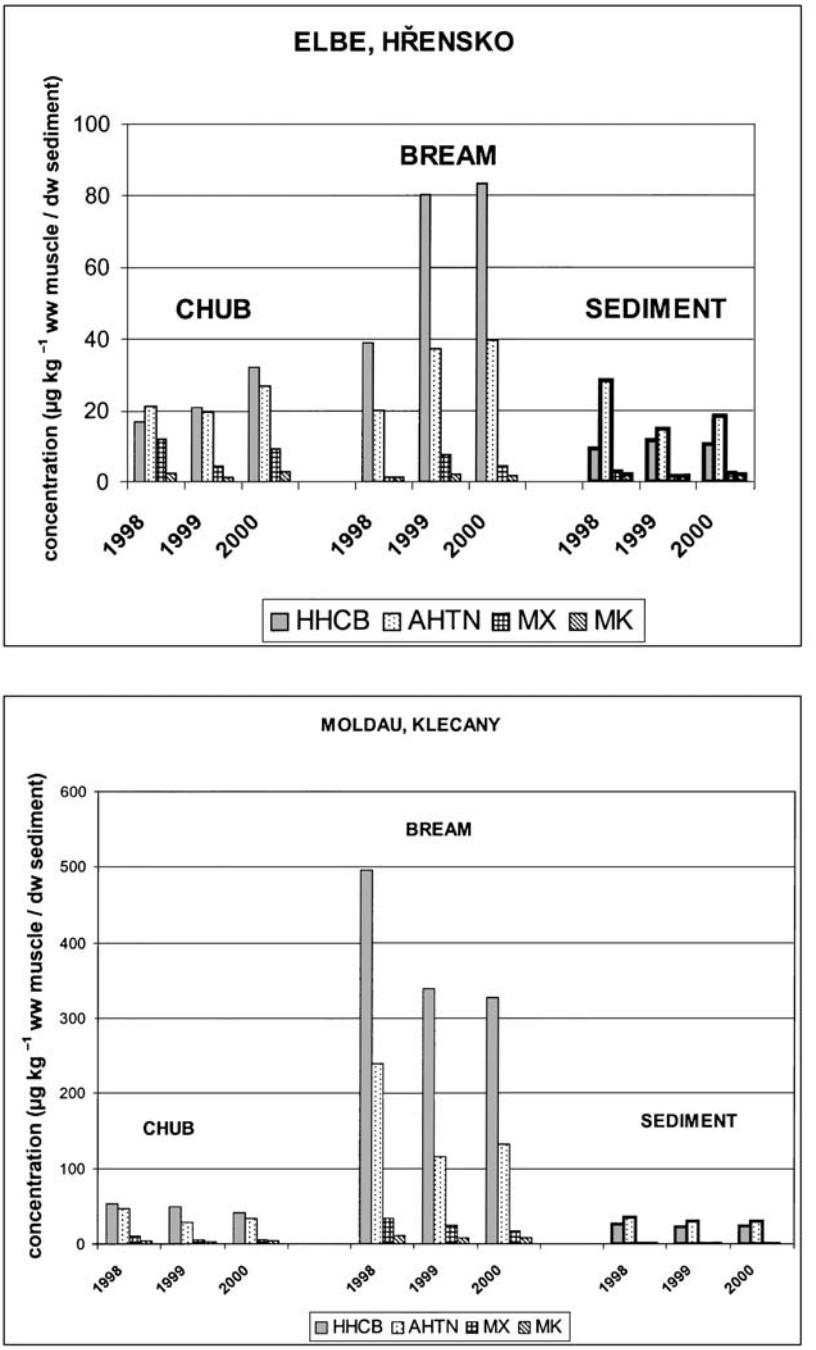

Fig. 3 Concentrations (medians) of synthetic musk compounds analysed in chub, bream and sediments collected in Hřensko (river Elbe) and Klecany (river Moldau) during monitoring program

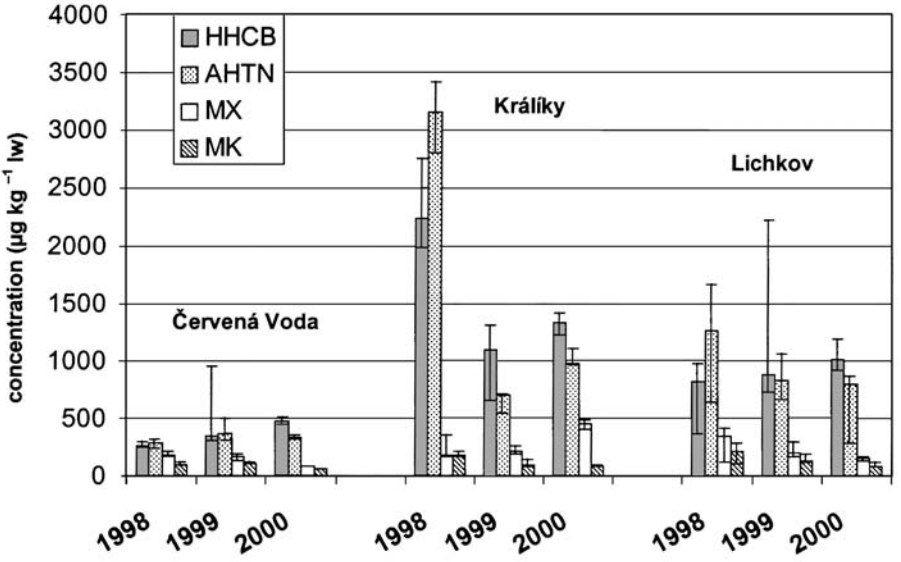

Fig. 4 Concentrations (medians) of synthetic musk compounds in trout from sampling sites at river Tichá Orlice

locality). On the other hand, samples from Králíky were in all the years distinctly more contaminated; extremely extensive pollution of trout by polycyclic musks was recorded in 1998. Interesting to note that, contrary to other years, concentrations of AHTN dominated in 1998 over HHCB. This rather untypical phenomenon was also observed in this year in the locality downstream of Lichkov. On the other hand, the levels of nitro musks in trout were at the same time relatively low. The same musks pattern was found in sediments which were, compared to other sampling sites, relatively extensively contaminated, specifically by polycyclic musks (probably not only due to high musks inputs but also because of relatively high content of organic matter which is responsible for binding of lipophilic pollutants). As one month prior to fish sampling local floods occurred at river Tichá Orlice in the year 1998, increased exposure of fish to mobilized sediments could have caused this unusual contamination pattern.

2.2 Fish as Biomonitor vs SPMD

Semipermeable membrane sampling device (SPMD) that was originally invented to study the bioavailability of hydrophobic organic chemicals such as PCBs and/or organochlorine pesticides to aquatic organisms [17] has nowadays become a widely used passive sampler finding a large range of applications including monitoring of freshwater pollution. Recently, SPMD has also been shown applicable in analysis of synthetic musks in polluted rivers and various reservoirs [10a, 13, 18]. To assess an informative/predictive value of data obtained by analysis of SPMD and to compare it with analyses of fish commonly used as biomonitor, a pilot

study was conducted within our monitoring program. In Fig. 5 there are shown concentrations of musks analysed in extracts recovered from the low-density polyethylene membranes that were deployed for three weeks in the river Moldau, locality Klecany.

In Fig. 5 there are also shown data obtained for several fish species collected here at the end of SPMD sampling period. As can be seen, under conditions applied in our experiments, the concentration range of polycyclic musks accumulated in a "virtual fish" represented by SPMD corresponded approximately to chub; on the other hand, as regards relative abundance of synthetic musks, the best similarity was recorded for bream. However, the levels of nitro musks determined in triolein isolated from semipermeable membrane bag were distinctly lower than those found in any of the fish species studied; the accumulation was low specifically for MK (MX/MK ratio was significantly higher in this kind of passive sampler than in fish). Very similar observations were obtained in other localities. Considering the physico-chemical properties of particular compounds,

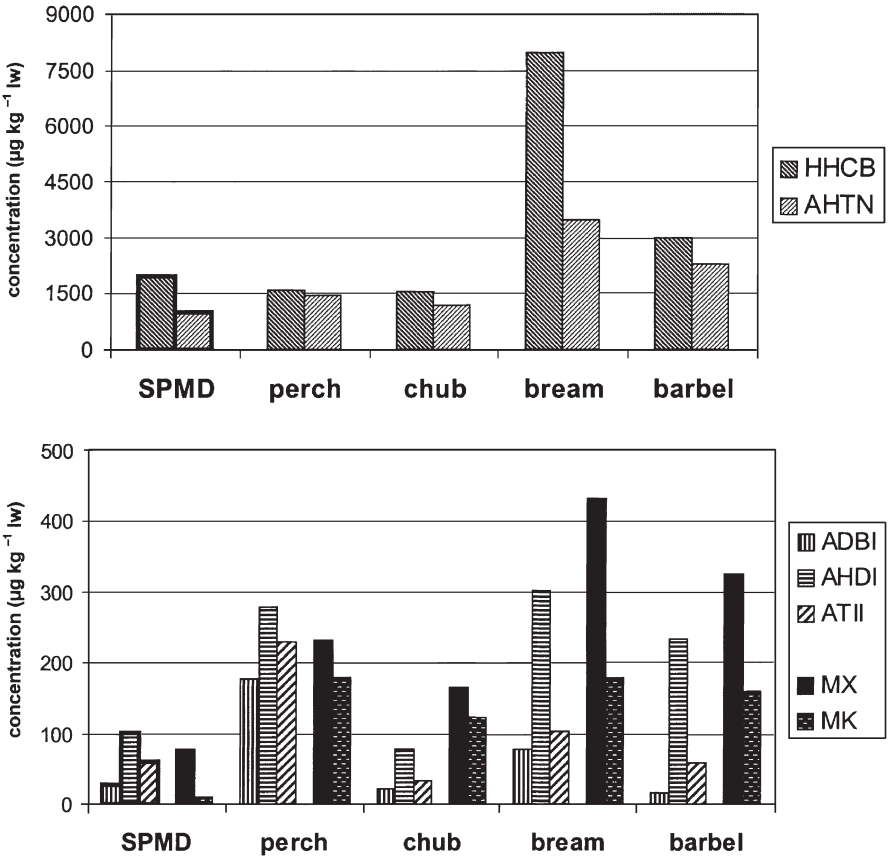

Fig. 5 Concentrations (medians) of synthetic musk compounds in perch, chub, bream and barbel, in comparison with those in SPMD (river Moldau, locality Klecany, sampling year 2000)

this phenomenon might be attributed to a lower lipophilicity of this class of musks (P_{ow} of MK and MX=4.2 and 4.9, respectively) [19] compared to polycyclic fragrances (e.g. P_{ow} of HHCB and AHTN=5.9 and 5.8, respectively) [7a]. In any case, it should be noted that musk patterns (relative abundance of individual compounds) in fish do not generally correlate with their physico-chemical properties. Significantly lower bioconcentration (uptake of chemical by absorption from water only) and bioaccumulation (combination of bioconcentration and food uptake) than expected on the basis of theory were invariably measured for polycyclic musks in several studies [7a, 16–20]. This discrepancy might be attributed to a strong metabolism taking place in these aquatic biota [10a, 13, 16]. For more details see the chapter of Biselli et al. in this monograph.

Regarding the SPMD uptake that corresponds to three weeks sampling period applied in our experiments, we assume it can be classified as a linear, integrative type of sampling, i.e. equilibrium was not approached. This assumption is in line with a recent study [8] reporting generally higher enrichment of polycyclic musk compounds in SPMDs after seven weeks of their deployment in the pond of a municipal sewage treatment plant compared to several fish species living here. Also the relative accumulation of MX and MK was higher as the result of significantly longer exposure period of SPMDs compared to our study. It should be noted that the plateau levels of AHTN and HHCB were rapidly reached (3–7 days of exposure) in bioconcentration tests employing bluegill sunfish [20]. However, as regards our study, it is rather difficult for us to speculate on the status of exposure phase of fish at the time of its catching because of different exposure conditions and wide inter-species variation as regards intake/excretion rates.

Simultaneously with synthetic musks, priority lipophilic contaminants such as PCBs and related persistent chlorinated aromatic compounds were determined in all examined samples. As illustrated in Fig. 6, the relative abundance of individual compounds composing the later group of pollutants as well as their analytical concentrations in trioleine after 21 days of SPMD deployment were

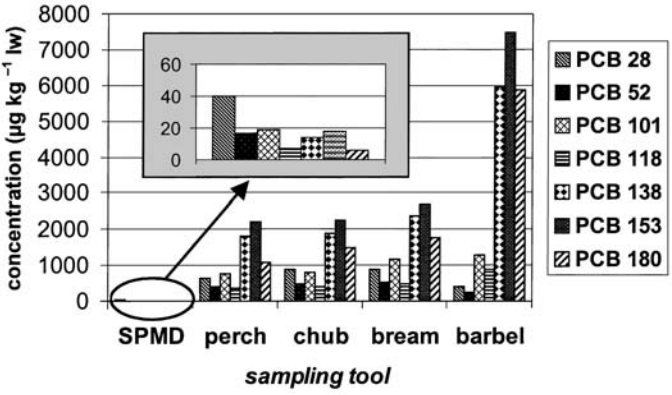

Fig. 6 Concentrations (medians) of PCBs in perch, chub, bream, and barbel in comparison with SPMD (river Moldau, locality Klecany, sampling year 2000)

mainly lower than those determined in fish. Poor agreement of the data for these chemicals obtained by this passive sampler and biomonitoring organism in spite of similar P_{ow} values to those of synthetic musks is mainly due to biomagnification occurring in fish in case of PCBs (this phenomenon obviously does not take place for musks). In any case, significantly longer deployment of SPMDs to attain equilibrium for PCBs would be needed.

2.3 Species-Dependent Pollution of Fish by Synthetic Musks

Remarkable differences in measured bioconcentration factors (BCFs) for individual musks among various fish species highlighted by several authors [7a, 8, 9, 21] have also been well documented in our study. As already shown in the foregoing Figures (see Figs. 3 and 5) and confirmed in Fig. 7, generally, the most extensive bioconcentration of musk compounds occurred in bream and/or in barbel while the lowest total musks levels were perpetually found in chub despite typically higher mean levels of lipid in this fish compared to those determined in other species.

Considering the whole data set obtained for polycyclic musks (including the minor compounds of this group) in the most abundant fish species, both the mean concentrations and median values were statistically higher in bream and/or barbel than in chub and/or perch regardless of the expression of results (either on wet weight or lipid weight). Regarding nitro musks, this trend was not so distinct. In any case, although differences in contamination of monitored localities existed, in all of them the relative amount of polycyclic musks was always higher

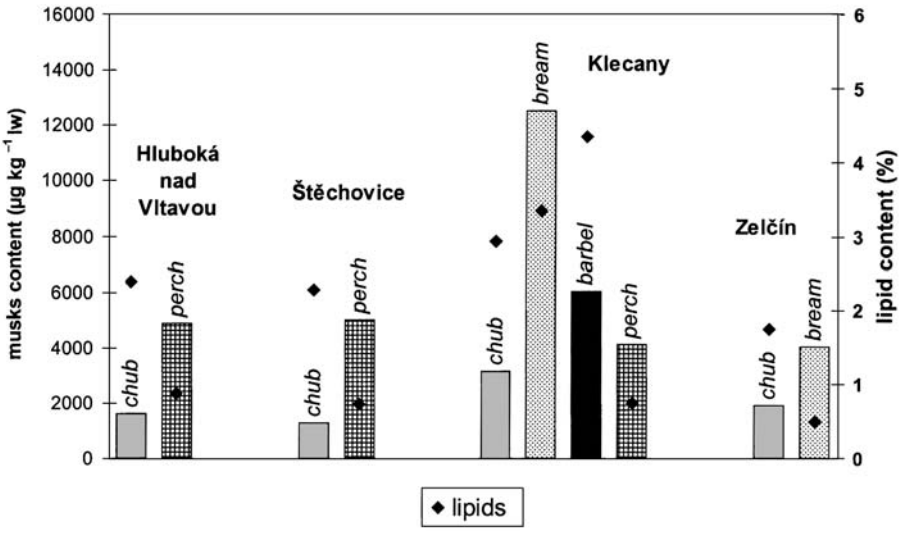

Fig. 7 Total concentrations of synthetic musk compounds (sum of examined analytes) in several fish species collected in sampling sites at river Moldau and mean values of lipid content (sampling year 2000)

in bream ranging between 90–95%; in chub their contribution to the total musks content was lower, in the range 75–86%.

Figure 8 illustrates typical patterns of analytes representing both groups of musks in fish from river Elbe, locality Hřensko.

In agreement with literature data [7a], the pattern of musks in muscle and liver of particular species were practically identical, the differences in analysed levels of musks were caused only by differing content of lipids in examined tissues. However, distinctive dissimilarities in relative abundance of HHCB and AHTN obviously due to the differences in the metabolization rate were recorded. While in chub the ratio of these dominating polycyclic musk compounds varied in a relatively narrow range typically slightly exceeding the value 1, in bream HHCB was clearly prevailing over AHTN, the ratio of major polycyclic musks ranged from approximately 1.5 up to almost 4. Considering the principles of pure bioconcentration mechanism [6] (which is theoretically simulated by SPMD sampling discussed above) as well as taking into account the data generated in presented study it might be speculated that less extensive metabolization of polycyclic musks, specifically of HHCB, occurs in bream, or vice versa a faster metabolism of AHTN. Our observations are in agreement with another study [21] concerned also with the examination of several fish species for musk levels: HHCB seems to be more prone to biotransformation compared to AHTN.

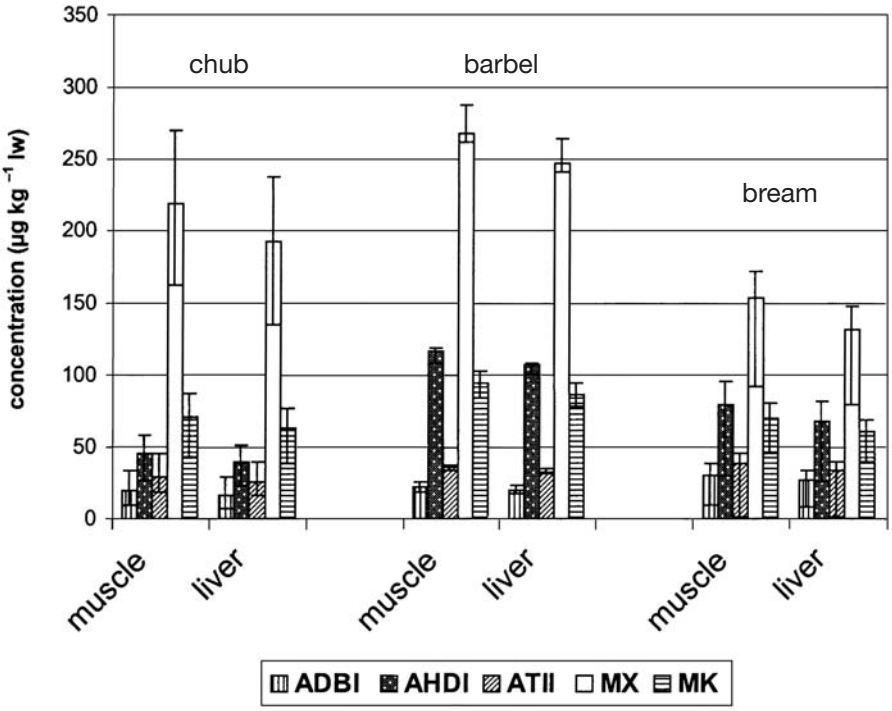

Fig. 8 Concentrations (medians) of synthetic musk compounds in muscle and liver of chub, barbel and bream (river Elbe, locality Hřensko, sampling year 2000)

3
Human Milk as Bioindicator: Set-Up of a Pilot Study

Human milk and adipose tissue is a widely employed bioindicator of the actual body burden of liphophilic and persistent compounds. While studies of a long-term exposure to organochlorine contaminants as well as studies of time-related trends in environmental contamination have already been conducted for several decades, the occurrence of musk compounds in these matrices were reported as late as at the beginning of the 1990s [22, 23a,b]. Regarding human exposure to synthetic musks, dietary intake seems to be negligible in this context, since meaningful contamination of food was found only in aquatic organisms (specifically freshwater fish) and not in other food of terrestrial animal origin. The main route of musks uptake is probably via dermal absorption due to frequent and intense dermal contact with fragrances contained in cosmetics and washed textiles [5, 23b, 25].

Before the pilot study presented here, no data were available in the Czech Republic for the assessment of human exposure by musks. In order to receive at least partial information, 59 milk samples were collected from nursing mothers (living but not necessarily born in Prague) by the Gynaecological Clinic which belongs to Medical Faculty, the Charles University, Prague. The manual sampling (milk expressed from the breast into a clean container) was conducted in accordance with WHO guidelines. Using a detailed questionnaire, relevant information on biological parameters such as age, dietary habits (specifically consumption of freshwater/marine fish), use of perfumed cosmetics, frequency of contacts with detergents, etc. were collated. Regarding the analytical procedure, isolation of analytes from milk samples was carried out according to AOAC procedure described in European norm 528 [26]. Isolated lipids (their content in milk ranged from 1.5 to 4.2 wt%) containing target analytes were processed in the same way as described above for fish samples. The limit of detection (LOD) was estimated at 10 μg kg^{-1} lw for all the analytes.

3.1
Synthetic Musks in Human Milk Samples Collected in Prague

In Table 8 the obtained results are summarized; for comparison, data from several similar German studies are listed too.

In all the Czech samples, concentrations of HHCB exceeded LOD; AHTN, MX and MK were determined in 90, 92 and 32% of samples, respectively. Although, based both on mean and median values, the concentration order HHCB>AHTN> MX>MK was found in the milk set, great differences existed among examined samples (Fig. 9); the distribution of analytes was not a Gaussian one.

In some of the milk samples the opposite concentration order within the particular group of synthetic musks was determined – either AHTN slightly exceeding HHCB (in 25% of cases) or MK was higher than MX (in three cases). The ratio of sum of polycyclic musks/sum of nitro musks ranged from 1 to 20. These facts clearly document a great diversity of exposure routes of individual donators. As regards the overall extent of breast milk contamination, distinctly higher levels of polycyclic musks were found in Czech samples when compared with

Table 8 Concentrations of synthetic musks (μg kg^{-1} lw) in human milk samples from Czech Republic, Prague; comparison with data obtained in German studies (references in square parentheses)

Parameter	Origin of study, Number of samples	HHCB	AHTN	MX	MK
Minimum	Czech Rep.	13	<10	<10	<10
	[22], n[a]=391	na[b]	na[b]	10	<10
	[23b], n[a]=23	na[b]	na[b]	40	10
	[5], n=5	16	11	10	5
	[27], n=55	na	na	10	nd[c]
Maximum	Czech Rep.	720	565	156	93
	[22], n=391	na	na	1220	240
	[23b], n=23	na	na	190	90
	[5], n=5	108	58	30	15
	[27], n=55	na	na	253	103
Median	Czech Rep.	149	67	42	30
	[22], n=391	na	na	70	30
	[27], n=55	na	na	40	10
Mean	Czech Rep.	214	112	53	39
	[22], n=391	na	na	100	40
	[27], n=55	na	na	41	10
Standard deviation (RSD %)	Czech Rep.	175 (82)	114 (101)	37 (70)	24 (61)
90% percentile	Czech Rep.	509	304	106	77
	[22], n=391	na	na	210	80

[a] n = number of samples.
[b] na = not analysed.
[c] nd = not detected.

data reported by Rimkus et al. [5]. On the other hand our results were more comparable to those (not shown in Table 8) reported by Eschke [24] in another study for two milk samples. In any case, very limited set of data available at present does not allow any general conclusions.

Contrary to polycyclic musks, relatively numerous studies have been concerned with the occurrence of nitro musks in this bioindicator [22, 23a,b, 27]. Levels of nitro musks determined in Czech samples corresponded or were slightly lower than values reported by German authors (Table 8), the MX/MK ratio seems to be rather lower (relative abundance of MX higher) in our samples (however, it is worth noting that maximum levels of MX reported in extensive study carried out nine years ago in Southern Bavaria [22] were higher by almost one order of magnitude). Whether continual drop in levels of nitro musks in Czech human milk occurs (the decrease of PCBs and other persistent contaminants was unequivocally documented one of our recent studies) in accordance with downward trend described in Germany within the period 1993–1996 [28] will reveal a follow-up study planned for the year 2003.

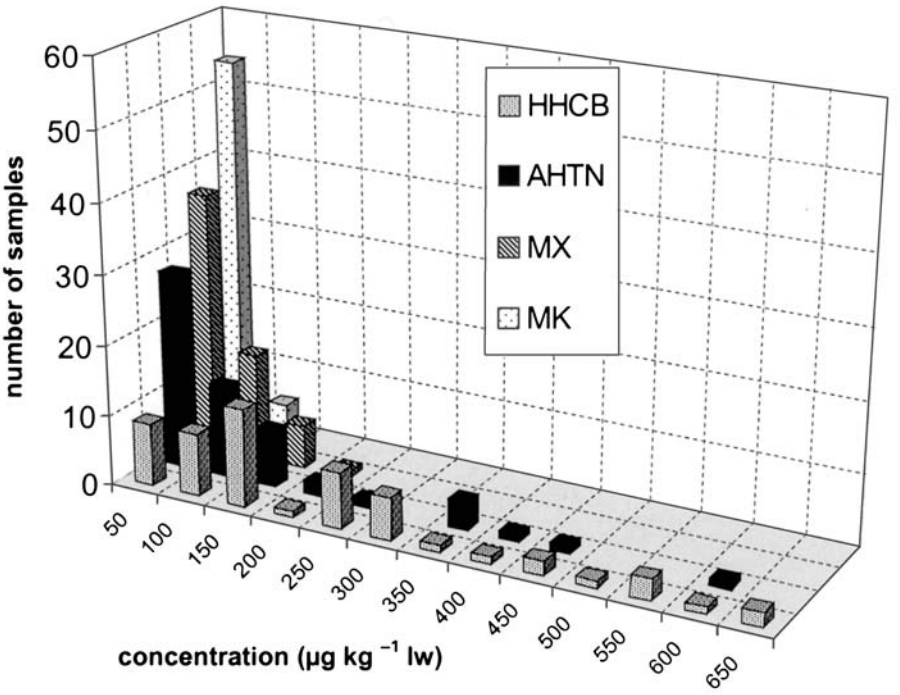

Fig. 9 Frequency distribution of synthetic musk compounds in 59 milk samples from Czech Republic (maximum value for HHCB [720 µg kg^{-1} lw] not shown here)

It should be noted, that statistical examination of all available information failed to prove any correlation between the musks levels and personal data of mothers obtained by the questionnaire. Due to generally low consumption of freshwater/marine fish (only 17% of responding mothers used to eat it each week), it might be speculated that dermal exposure is dominating way of synthetic musk intake in examined population. As declared by many other authors concerned with this issue, more research is obviously needed in this field to take effective measurements aimed at minimizing of human contamination.

4 Conclusions

Polycyclic musks as well as nitro musks were shown to be ubiquitous contaminants of aquatic ecosystems in Czech Republic. Both sediments and fish collected at monitored sampling sites of river Elbe and its tributaries Moldau and Tichá Orlice contained the same representatives of polycyclic fragrances and nitro musks as reported in other European studies. In accordance with them, the former group of musks was distinctly dominating, the maximum concentrations in fish (sampled from river Moldau downstream of Prague) were as high as 10^4 µg kg^{-1} lw (the highest levels of MX were 10^3 µg kg^{-1} lw). The relative abun-

dance of particular synthetic musks were matrix-dependent, while in fish the typical concentration order of major musks was HHCB>AHTN⩾MX>MK; in sediments the typical pattern was AHTN>HHCB⩾MX=MK. In any case, significant correlation (r^2>0.8) between the contamination of sediments and content of musks in fish collected at particular locality was proved. With respect to a very similar pattern of synthetic musks in respective fish species regardless of the extent of particular locality pollution, it seems there are not distinct differences either in sewage treatment technology or the sources of waste contamination (the use of scented products).

Although any of the examined fish species may be employed as a relevant bioindicator of the presence of sewage waters in respective aquatic ecosystem, the best suited fish for this purpose seems to be bream because of its highest bioconcentration potential. This phenomenon might be attributed to low intensity of musk metabolization compared to other fish species involved in the study. As regards the sampling of fish for monitoring of musks, its age/size is not important since only bioconcentration mechanism is responsible for the uptake of these water pollutants.

An alternative sampling technique represented by SPMD was shown to be very well suited for monitoring of polycyclic musks (the levels in this "virtual fish" correspond to lipophilicity of particular substances dissolved in water). Lower sensitivity of nitro musks detection, specifically of MK, has to be taken into account due to its higher polarity.

Results obtained by analysis of breast milk samples showed extensive exposure of some donators by these synthetic fragrance compounds. However, no generalization with respect to the route of exposure could be drawn on the basis of collated information. A follow-up study focussing on clarification of this issue will be initiated.

Acknowledgements We gratefully acknowledge the financial support provided by the Ministry of Environment, project VaV 340/1/01 (fish samples) and the Ministry of Education, Youth and Sports of the Czech Republic, project MSM 223300004 (breast milk samples).

5 References

1. a) Rimkus G, Wolf M (1993) Dtsch Lebensm-Rundsch 89:171; b) Rimkus G, Wolf M (1995) Chemosphere 30:641
2. a) Eschke H-D (1994) Ruhrwassergüte, Ruhrverband, Essen, Germany, p 62; b) Eschke H-D, Traud J, Dibowski H-J (1994) UWSF-Z Umweltchem Ökotox 6:183; c) Eschke H-D, Traud J, Dibowski H-J (1994) Vom Wasser 83:373; d) Eschke H-D, Dibowski H-J, Traud J (1995) UWSF-Z Umweltchem Ökotox 7:131; e) Eschke H-D (1996) Ruhrwassergüte, Ruhrverband, Essen, Germany, p 86
3. Lagois U (1996) gwf Wasser Abwasser 137:154
4. de Boer J, Brinkman UAT (1994) Trends Anal Chem 13:397
5. Rimkus GG, Wolf M (1996) Chemosphere 33:2033
6. Geyer HJ, Rimkus GG, Scheunert I, Kaune A, Kettrup A, Zeeman M, Muir DCG, Hansen LG, Mackay D (2000) In: Beek B (ed) Bioaccumulation, new aspects and developments. Handbook of environmental chemistry, vol 2, part J. Springer, Berlin Heidelberg New York, p 1
7. a) Rimkus GG (1999) Toxicol Lett 111:37; b) Rimkus GG, Wolf M (1997) Lebensmittelchemie 51:94; c) Rimkus GG, Brunn H (1996) Ernährungs-Umschau 43:442

8. Gatermann R, Biselli S, Hühnerfuss H, Rimkus GG, Hecker M, Karbe L (2002) Arch Environ Contam Toxicol 42:437
9. Rimkus GG, Gatermann R, Hühnerfuss H (1999) Toxicol Lett 111:5
10. a) Gatermann R, Hühnerfuss H, Rimkus G, Attar A, Kettrup A (1998) Chemosphere 36:2535; b) Gatermann R, Hellou J, Hühnerfuss H, Rimkus G, Zitko V (1999) Chemosphere 38:3431
11. Herren D, Berset JD (2000) Chemosphere 40:565
12. Draisci R, Marchiafava C, Ferretti E, Palleschi L, Catellani G, Anastasio A (1998) J Chromatogr A 814:187
13. Biselli S, Gatermann R, Rimkus GG, Hühnerfuss H, Kallenborn R (2000) Third SETAC World Congress, Brighton, UK, 24–29 May 2000. Proceeding abstract 3E/p026
14. Rimkus GG, Butte W, Geyer HJ (1997) Chemosphere 35:1497
15. Winkler M, Kopf G, Hauptvogel T, Neu T (1998) Chemosphere 37:1139
16. Fromme H, Otto T, Pilz K (2001) Water Res 35:121
17. Huckins JN, Petty JD, Prest HF, Clark RC, Alvarez DA, Orazio CE, Lebo JA, Cranor WL, Johnson BT (2002) A guide for the use of semipermeable sampling devices (SPMDs) as samplers of waterborne hydrophobic organic contaminants. American Petroleum Institute, Publication No 4690
18. Schwartz S, Berding V, Matthies M (2000) Chemosphere 41:671
19. Tas JV, Balk F, Ford RA, van de Plassche EJ (1997) Chemosphere 35:2973
20. Balk F, Ford RA (1999) Toxicol Lett 111:57
21. Franke S, Meyer C, Heinzel N, Gatermann R, Hühnerfuss H, Rimkus GG, König WA, Francke W (1999) Chirality 11:795
22. Liebl B, Ehrenstorfer S (1993) Chemosphere 27:2253
23. a) Rimkus G, Wolf M (1993) Dtsch Lebensm-Rundsch 89:103; b) Rimkus G, Rimkus B, Wolf M (1994) Chemosphere 28:421; c) Rimkus GG, Wolf M (1996) Chemosphere 33:2033
24. Eschke H-D, Dibowski H-J, Traud J (1995) Dtsch Lebensm-Rundsch 91:375
25. Müller S, Schmid P, Schlatter C (1996) Chemosphere 33:17
26. CEN (1996) Fatty food – determination of pesticides and PCBs, part 2: extraction of fat, pesticides and polychlorinated biphenyls (PCBs), and determination of fat content. Paragraph 6.1.1 AOAC extraction, EN 152822. CEN, Brussels
27. Ott M, Failing K, Lang U, Schubring C, Gent H-J, Georgii S, Brunn H (1999) Chemosphere 38:13
28. Weizenegger W, Kypke K, Fetteroll B, Hahn J, Dsohnius E, Feucht M (1998) Dtsch Lebensm-Rundsch 94:103