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Levels, Tissue Distribution, and Age-Related Accumulation of Synthetic Musk Fragrances in Chinese Sturgeon (*Acipenser sinensis*): Comparison to Organochlorines

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Chinese sturgeon (*Acipenser sinensis*) was listed as a Grade I protected animal in China in 1989, and the observed intersexual phenomenon and sex ratio deviation have suggested that chemicals have posed a risk as environment pollutants. This study analyzed seven musk fragrances in liver, muscle, heart, gonad, stomach, intestines, adipose, gill, pancreas, kidney, gallbladder, and roe from 13 female Chinese sturgeons, and the toxicokinetic behavior of musks were studied and compared with some organochlorines. Of the seven musks, HHCB, AHTN, and musk xylene were detected, and the highest concentrations were observed in adipose tissue: from 33.7 to 62.1 ng/g wet weight (ww), from 1.0 to 5.4 ng/g ww, and from 1.1 to 13 ng/g ww, respectively. Similar to the tissue distribution of DDTs and HCB, musks were detected frequently in high lipid content tissues such as roe, adipose, and liver, suggesting that tissue distribution of musks is controlled by the affinity to lipids. The concentration ratios based on lipid weight between roe and adipose were estimated to be 0.47 for HHCB, 0.58 for AHTN, and 0.51 for musk xylene, and those for the total DDTs and HCB were 0.27 and 0.61, which were relatively low compared with mammals. Relatively high concentrations of *p,p′*-DDE (68.4−449 ng/g ww) were detected in 10 of total 11 samples, which would cause the feminization of Chinese sturgeons during embryonic development. It was found that lipid-corrected concentrations of HHCB, AHTN, *p,p′*-DDE, and *p,p′*-DDD increased with age in female sturgeon, of which the trends were similar to those in fishes and different from those in mammals.

Introduction

Synthetic musk compounds, including nitro musks and polycyclic musks, have recently been recognized as important organic contaminants in aquatic environments (*1*). Musk xylene was an inducer of mouse CYP2B enzymes, an amine metabolite of musk xylene is a mechanism-based inactivator of mouse CYP2B10 according to in vivo assays (*2*), and this compound was carcinogenic based on a long-term exposure toxicity study in mice (*3*). Recent studies reported that some nitro musks such as musk ketone, musk xylene, and their metabolites, 7-acetyl-1,1,3,4,4,6-hexamethyltetrahydeonaphthalene (AHTN) and 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[*g*]-2-benzopyran (HHCB), elicited estrogenic activity in both in vitro and in vivo assays (*4*−*6*).

Synthetic musk fragrances are used extensively in a variety of personal-care products as surrogates for natural musk, in additives for cigarettes and fish baits, and in technical products such as herbicide formulations and explosives. The worldwide production of nitro musks and polycyclic musks was estimated to be 2500 tons in 1988 (*7*) and more than 6000 tons in 1996 (*1*), respectively. In 1981, nitro musks were first identified in the aquatic ecosystem in Japan (*8*). In the subsequent investigations on the occurrence of musks in the environment, musk compounds were detected in the atmosphere, surface water, sewage, and sediments in Europe and North America, demonstrating that musks are ubiquitous in the environment (*1*, *9*, *10*). Because of their properties of lipophilicity (log K_{ow} of polycyclic musks, 4.5−6.3 (*1*); log K_{ow} of nitro musks, 3.8−4.4 (*11*)), musk fragrances are bioaccumulative in aquatic biota, which was supported by the residues in fish and invertebrates from both freshwater and marine environment (*7*, *9*, *12*, *13*). The bioaccumulation factors of HHCB, AHTN, musk xylene, and musk ketone were estimated to be 20−1584, 40−670, 290−40 000, and 60−1380, respectively (*14*−*16*), and they were found to be species-specific (*13*, *16*). Besides the ubiquitous occurrence of musk in fish and invertebrates, musks have also been detected in high trophic marine organisms such as sharks, seals, and dolphins (*17*, *18*).

Chinese sturgeon (*Acipenser sinensis*) is predatory fish and feed at the top of the aquatic food web (*19*). As one of the 25 extant sturgeon species protected under the convention of the International Trade of Endangered Species, Chinese sturgeon is listed as a Grade I protected animal in *China Red Data Book of Endangered Animals* (*20*) due to the rapid decline in its population. While the declining population was attributed to overfishing and dam construction by the public and the Chinese government (*21*, *22*), the risk of environmental pollutants such as endocrine-disrupting chemicals has been neglected (*23*). Recent studies have clarified the increase of female−male sex ratios (from 0.79 in 1981−1993 (*21*) to 5.9 in 2003−2004 (*24*)), the declining activity of sperm, and intersexual phenomenon (*21*, *25*), implying that environmental pollution poses a risk to Chinese sturgeon.

In this study, we analyzed seven musk fragrances (HHCB, AHTN, 4-acetyl-1,1-dimethyl-6-*tert*-butylindan (ADBI), musk ketone, musk xylene, musk ambrette, musk tibeten) in liver, muscle, heart, gonad, stomach, intestines, adipose, gill, pancreas, kidney, gallbladder, and roe from 13 Chinese sturgeons, and then the tissue distribution, maternal transfer, and age-related accumulation of musk fragrances were estimated and compared with organochlorines, including hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its related chemicals.

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TABLE 1. Details of Chinese Sturgeon Samples Used in This Study

sample code	date of collection	tissue[a]	age (year)	wt (kg)	length (cm) total length	length (cm) body length
A0466	2003	L, M, H, Go, St, I, A, Gi, K	24	254	339	285
A0406	2004	R, L, M, H, Go, St, P	18	174	297	245
A0410	2004	R, L, M, H, Go, St, I, A, Gb	17	140	288	246
A0412	2004	R, L, M, H, Go, St, I, Gi, P	24	230	334	287
A0414	2004	R, L, M, I, A, Gi	25	263	337	285
A0408	2004	R	22	230	312	258
A0447	2005	R, L, M, H, Go, I, Gi,	19	192	303	247
A0445	2005	L, M, H, Go, I, A, Gi	18	187	285	237
A0403	2005	R	24	260	338	280
A0444	2005	R	23	224	320	270
A0452	2005	R	23	207	322	282
A0449	2005	R	22	252	327	275
A0500	2005	R	22	227	317	261

[a] L: liver; M: muscle; H: heart; Go: gonad; St: stomach; I: intestines; A: adipose; Gi: gill; P: pancreas; K: kidney; Gb: gallbladder; R: roe. All sturgeons were female.

Materials and Methods

Sample Collection. Chinese sturgeon are typical anadromous fish. They live in the sea until the initial reproduction of fish at an average age of ~14.3 years (*21*). At the end of spring (June−July) every year, maturing adults stop feeding, leave the ocean, and ascend the main channel of the Yangtze River to spawning grounds. Prespawners (sturgeons with immature gonads) mature for ~1 year in the Yangtze River to reproduce during the spawning season (early October−early November) of the next year and then return to the sea (*21*, *26*). Offspring arrive in the estuary of the Yangtze River along the main channel of the river the next May. Since the migration route of Chinese sturgeon was blocked by the Gezhou Dam in 1981, the population of fish has declined sharply (*22*), and artificial propagation was begun to conserve this endangered species (*26*). Because this sturgeon is listed as a Grade I protected animal (the highest level of protection) in China, the capture of Chinese sturgeon only for scientific purposes must be authorized by the Ministry of Agriculture of the People's Republic of China, and the number is limited: eight to ten every year in recent years. After artificial propagation, the live sturgeon must be released into the river under the supervision of the local government, but some sturgeon would die. In this study, the roes before artificial fecundation and tissues from the dead fish were collected during 2003−2005 and frozen immediately at −20 °C until analysis. Ages of fish were determined by counting growth layers in clreithrum, as described previously (*19*, *21*, *24*). Details of the samples analyzed in this study are shown in Table 1.

Sample Preparation. Approximately 0.5−10 g of tissues (about 0.5−1 g for adipose, 1−2 g for roe and liver, and 5−10 g for other tissues) mixed with ~20 g of Na_2SO_4 were spiked with surrogate standard (phenanthrene-d_{10}, PCB 121, and PCB 198) and Soxhlet extracted for 24 h using 250 mL of dichloromethane/methanol (7:3 v/v) mixture solution. Twenty percent of the extract was used to determine the lipid percentage. The extracts were rotated to dryness and heated at 65 °C for ~30 min, and lipid amounts were determined gravimetrically. The remaining 80% of the extract was subjected to acetonitrile partitioning to remove lipid as described previously (*27*). The extracts were rotoevaporated and reconstituted by 1 mL of hexane and then added to a separatory funnel containing 50 mL of hexane-saturated acetonitrile and 20 mL of hexane. After the solution was shaken for 5 min and partitioned, the acetonitrile layer was collected. This procedure was repeated, and a total of 100 mL of hexane-saturated acetonitrile was collected and transferred to a 1-L separation funnel containing 500 mL of 5% NaCl solution and 50 mL of hexane. After the solution was shaken, the water layer was removed and the hexane was collected. These procedures were repeated, and a total of 100 mL of hexane was obtained. About 10 g of Na_2SO_4 was used to remove moisture, and then the hexane was concentrated to exactly 50 mL. Half of the hexane was stored, and the rest was concentrated to ~1 mL and passed through a glass column (10-mm i.d.) containing 10 g of 5% H_2O-deactivated neutral Al_2O_3 (200 mesh size, Shanghai Ludu Chemicals, Shanghai, China) for cleanup. This column was eluted with 30 mL of high-purity hexane and 30 mL of hexane/dichloromethane (3:1 v/v). The eluant was dried and dissolved in 0.2 mL of hexane for gas chromatography/mass spectrometry (GC/MS) analysis. The information about chemicals and standards and chemical analysis is provided in Supporting Information.

Quantitation and Quality Assurance Quality Control. All equipment rinses were done with methanol to avoid sample contamination, and a procedural blank was analyzed with every set of seven samples. Quantification of the musks, HCB, HCHs, and DDTs in biota samples was carried out by using relative response factors determined from calibration standard runs. To automatically correct the losses of analytes during extraction or sample preparation, as well as for variations in instrument response from injection to injection, surrogate standards were used in this study. Phenanthrene-d_{10}, PCB 121, and PCB 198 do not exist in the environment and were used as surrogate standard for musks and organochlorines, respectively, in previous studies (*18*, *28*−*30*). In the present study, HCBs, HCB, *p*,*p*′-DDMU, *o*,*p*′-DDD, *p*,*p*′-DDD, *o*,*p*′-DDE, and *p*,*p*′-DDE were quantified in sample extracts relative to PCB 121, and *o*,*p*′-DDT, *p*,*p*′-DDT were quantified relative to PCB 198. Phenanthrene-d_{10} was used as a surrogate standard for musks as exemplified in previous papers (*17*, *18*), because the surrogate shows similar physicochemical properties such as logarithm octanol−water partition coefficient (4.57 for phenantharene (*31*) and 4−6 for musks (*1*, *11*)) and retention time on GC column with those of musks.

For calibration purposes, four different concentrations of compounds (10, 25, 50, and 100 μg/L for musks, *o*,*p*′-DDT, and *p*,*p*′-DDT; 5, 20, 50, and 100 μg/L for HCB, HCHs, and other DDTs) and their respective surrogate standards were analyzed by GC/MS to obtain relative response factors for all standards. For sample analysis, the procedure described above was validated for recovery experiment by choosing a roe sample (A0410) with low pollution level as spiked sample. Analyte addition was made with the criterion of at least three times the original concentration. The six replicate roe samples, spiked with 80 ng of musks, 200 ng of DDTs and HCHs, and 600 ng of HCB, and one roe sample (matrix blank sample) were analyzed to determine the general recovery rate. The recoveries for spiked samples were 89−120% for HCHs and HCB, 99−113% for DDTs, 74−111% for musks, 70−102% for PCB 121, 66−89% for PCB 198, and 70−94% for phenanthrene-d_{10}. Since we found that lipid amount was the main factor affecting the recovery for analysis because of the emulsion during the extraction, the amount of a sample for each tissue was controlled to make the lipid amount lower than that in the spiked roe samples. For all the biota samples for analysis, the recoveries of PCB 121, PCB 198, and phenanthrene-d_{10} were 84 ± 14, 70 ± 15, and 85 ± 15%, respectively.

For HHCB and AHTN, the blank samples were used to correct the sample value due to the ubiquity of these chemicals, and the limits of quantitation (LOQs) were set to be the three concentrations in the blank samples. The

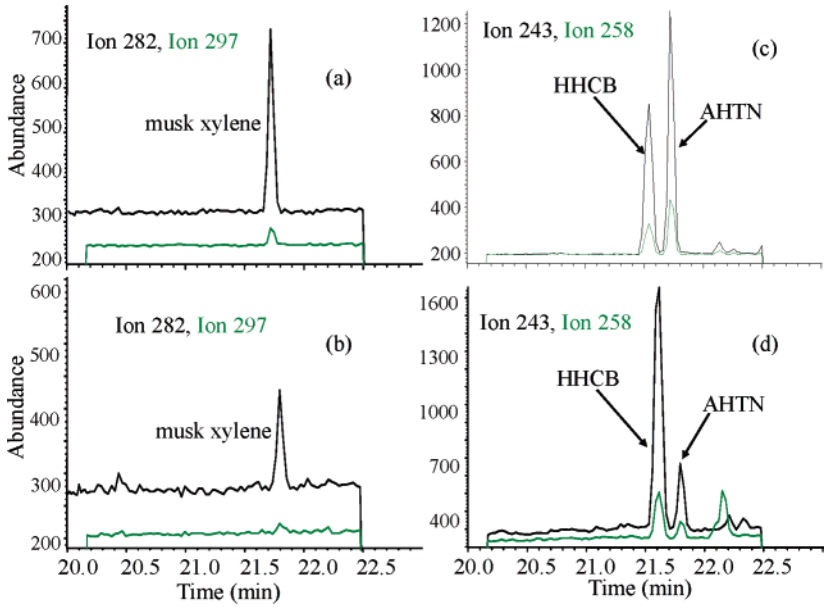

FIGURE 1. GC/MS chromatograms of HHCB, AHTN, and musk xylene in adipose samples of Chinese sturgeon. (a) Musk xylene standards; (b) musk xylene in samples; (c) HHCB and AHTN standard mixture; (d) HHCB and AHTN in samples; identification ions were *m/z* 243 and 258 for HHCB and AHTN and *m/z* 282 and 297 for musk xylene.

detection limits for HHCB, AHTN, ADBI, musk ambrette, musk xylene, musk tibeten, and musk ketone were 1.3, 0.5, 0.2, 0.5, 0.5, 0.2, and 0.5 ng/g, respectively. And the detection limits were 0.05 ng/g for *p,p′*-DDMU, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE, and *p,p′*-DDE, 0.2 ng/g for *o,p′*-DDT and *p,p′*-DDT, 0.07 ng/g for HCB, and 0.1 ng/g for HCHs.

Results and Discussion

Residue Levels. Figure 1 shows the GC/MS chromatograms of HHCB, AHTN, and musk xylene in an adipose sample (A0410 in Table 1). Of the seven musk fragrances, only HHCB, AHTN, and musk xylene were detected mainly in liver, adipose, and roe (Table 2), and the concentrations of other musk fragrances were all below the detection limit. While HHCB was detected in three liver samples ($n = 7$; <LOQ 15.9 ng/g wet weight (ww), two gonad samples ($n = 6$; <LOQ 4.0 ng/g ww), and two gill samples ($n = 5$; <LOQ 3.5 ng/g ww), it was detected in all adipose samples ($n = 4$; 33.7−62.1 ng/g ww) and all roe samples ($n = 11$; 10.2−44.1 ng/g ww). Concentrations of HHCB were detected only in one kidney (5.3 ng/g ww) and one gallbladder (6.5 ng/g ww). AHTN was detected in 2 liver samples ($n = 7$; <LOQ 0.9 ng/g ww), 10 roe samples ($n = 11$; <LOQ 5.4 ng/g ww), and all adipose samples (1.0−5.4 ng/g ww), musk xylene was only found in all adipose (1.1−13 ng/g ww) and roe samples (0.6−4.6 ng/g ww), and these concentrations were relatively lower than those of HHCB. The concentrations of the three detected musks were all lower than those reported in fish collected from some European rivers (*7*, *13*), but the HHCB and AHTN concentrations in adipose of Chinese sturgeon were higher than those in blubber of dolphins and whales on the coast of Florida in the United States (*17*), and the HHCB concentration in liver was comparable to those in liver tissues of hammerhead sharks in Japan (*18*). Previous investigations show that HHCB, AHTN, musk xylene, and musk ketone have been the most commonly found synthetic musks in environmental samples (*1*, *7*, *9*). Interestingly, polycyclic musks, especially HHCB and AHTN, were dominant in fish in Europe. Nitro musks, especially musk ketone, dominated in fish in Canada (*7*), and only HHCB is the most frequently detected compound in marine mammals and shark in Japan (*18*). This reflects the application characteristics of different areas. Our study showed that HHCB was the main fragrance, and the levels of musk xylene was comparable to that of AHTN in Chinese sturgeon samples, which corresponds to the increase in the production of musk xylene in China (*32*).

As for organochlorines, *p,p′*-DDE (accounting for 54−100% of total DDTs), *p,p′*-DDD (accounting for 0−46% of total DDTs), and HCB were detected in all of the samples, but no HCHs were detected in any of the samples (Table 2). HCB concentrations (224−2600 ng/g lipid weight (lw)) in Chinese sturgeon muscle were comparable to those in sturgeons collected from the Caspian Sea (*29*). Concentrations of DDTs ranged from 394 to 15900 ng/g lw in muscle of Chinese sturgeon, which were comparable to those (200−20 000 ng/g lw total DDTs) in four sturgeon species collected from the Caspian Sea (*33*) and those (1900 ng/g lw total DDTs) in White sturgeon from San Francisco Bay (*34*). The highest concentration of *p,p′*-DDE in roe of Chinese sturgeon was 449 ng/g ww, which was close to that (780 ng/g ww) in roe of shovelnose sturgeon in the Mississippi River, for which intersexual characteristics were reported (*35*). It is proven that *p,p′*-DDE is a androgen receptor antagonist, and the concentration of *p,p′*-DDE required to inhibit androgen receptor transcriptional activity in monkey kidney CV-1 cell culture was 63.6 μg/L (*36*), which is lower than those in 10 of a total 11 roe samples (68.4−449 ng/g ww). This suggested that the increasing feminization in Chinese sturgeon in the last 20 years would reflect antiandrogenic activity of *p,p′*-DDE, acting at the level of the androgen receptor.

Sturgeon roe are of extremely great commercial value in the world. In the present study, HHCB, AHTN, musk xylene, HCB, and DDTs were detected in all roe samples ($n = 11$). It was found that detected musks by lipid weight correlate well with each other ($r^2 = 0.70-0.92$, $p = <0.001-0.001$). Similar correlative relationships were also found between concentrations of DDTs and those of HCB ($r^2 = 0.5636$, $p = 0.008$). These results suggested the coexposure of Chinese sturgeons to HHCB, AHTN, and musk xylene and to DDTs and HCB, respectively.

Tissue Distribution. Figure 2 shows a comparison of the tissue distribution of HHCB with those of ∑DDTs and HHCB (ng/g ww) in different tissues ($n > 3$) of Chinese sturgeon. It was found that the concentrations of HHCB were high in adipose (33.7−62.1 ng/g ww), roe (10.2−44.1 ng/g ww), and liver (<LOQ 15.9 ng/g ww), and the lipid contents of the

TABLE 2. Concentrations of Musks and Organochlorines in Different Tissues (ng/g Wet Weight) of Chinese Sturgeon[a]

	lipid(%)		HHCB	AHTN	musk xylene	*p,p′*-DDMU	*o,p′*-DDE	*p,p′*-DDE	*o,p′*-DDD	*p,p′*-DDD	*o,p′*-DDT	*p,p′*-DDT	HCB
liver $n = 7$	12.2 ± 7.4	minimum	<LOQ	<LOQ		<LOQ		1.7		1.2		<LOQ	1.8
		maximum	15.9	0.9	<LOQ	1.5	<LOQ	1800	<LOQ	23.9	<LOQ	13.2	204
		mean ± SD	4.0 ± 6.3	0.22 ± 0.39		0.7 ± 0.7		349 ± 646		10.3 ± 8.4			98.0 ± 70.8
muscle $n = 7$	1.9 ± 1.2	minimum						3.2	<LOQ	0.2		<LOQ	3.0
		maximum	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	71.1	0.2	1.7	<LOQ	2.7	18.7
		mean ± SD						23 ± 22.4		1.1 ± 0.7			10.3 ± 5.0
heart $n = 6$	4.2 ± 1.7	minimum				<LOQ	<LOQ	3.9		0.4			3.1
		maximum	<LOQ	<LOQ	<LOQ	0.2	0.1	92.0	<LOQ	3.1	<LOQ	<LOQ	14.1
		mean ± SD				0.1 ± 0.05		25.5 ± 32.9		1.4 ± 1			9.9 ± 4.0
gonad $n = 6$	3.6 ± 1.7	minimum	<LOQ			<LOQ	<LOQ	3.7	<LOQ	1.0			12.5
		maximum	4.0	<LOQ	<LOQ	0.3	0.2	157.6	0.9	12.3	<LOQ	<LOQ	121.0
		mean ± SD						83 ± 75.0	0.3 ± 0.3	3.7 ± 4.3			34.6 ± 42.8
stomach $n = 4$	1.3 ± 0.4	minimum				<LOQ		0.4		0.2			2.1
		maximum	<LOQ	<LOQ	<LOQ	0.1	<LOQ	27.0	<LOQ	0.7	<LOQ	<LOQ	5.9
		mean ± SD						8.1 ± 12.7		0.4 ± 0.2			4.2 ± 1.8
intestines $n = 6$	2.8 ± 1.6	minimum				<LOQ		2.6		0.4			1.6
		maximum	<LOQ	<LOQ	<LOQ	0.1	<LOQ	142	<LOQ	4.1	<LOQ	<LOQ	25.7
		mean ± SD						30.2 ± 55.1		1.9 ± 1.5			10.2 ± 8.5
adipose $n = 4$	66 ± 18	minimum	33.7	1.0	1.1	1.0	0.9	138.0	0.7	7.5	3.3	12.9	96.1
		maximum	62.1	5.4	13.0	2.0	1.7	1130.0	1.9	28.2	20.4	480.0	493.0
		mean ± SD	45.8 ± 12.0	2.4 ± 2.1	4.3 ± 5.8	1.3 ± 0.5	1.3 ± 0.4	620 ± 405	1.4 ± 0.5	19.2 ± 9.9	9.6 ± 7.6	196 ± 212	358 ± 183
gill $n = 5$	2.4 ± 0.6	minimum	<LOQ			<LOQ	<LOQ	4.0	<LOQ	0.5			3.0
		maximum	3.5	<LOQ	<LOQ	0.3	0.3	100.0	0.3	1.6	<LOQ	<LOQ	42.6
		mean ± SD						48.8 ± 44.4	0.11 ± 0.1	1 ± 0.48			15.6 ± 16.2
pancreas $n = 2$	6.8	minimum				<LOQ		109.0		0.6			16.4
		maximum	<LOQ	<LOQ	<LOQ	0.2	<LOQ	134	<LOQ	3.3	<LOQ	<LOQ	139
kidney $n = 1$	31.5		5.3	<LOQ	<LOQ	0.4	0.6	121	<LOQ	5.0	<LOQ	<LOQ	17.4
gallbladder $n = 1$	23.0		6.5	<LOQ	<LOQ	<LOQ	<LOQ	44.0	<LOQ	4.6	<LOQ	<LOQ	12
roe $n = 11$	33.7 ± 9.8	minimum	10.2	<LOQ	0.6	0.1	<LOQ	29.3	0.2	1.8	0.2	2.1	45.4
		maximum	44.1	5.4	4.6	1.3	1.3	449	0.9	11.0	3.3	21.7	525
		mean ± SD	27.6 ± 11.1	2.2 ± 1.7	2.4 ± 1.7	0.6 ± 0.4	0.6 ± 0.3	179 ± 123	0.6 ± 0.3	6.5 ± 3	1.7 ± 1.2	11 ± 6.7	199 ± 152

[a] The detection limits for HHCB, AHTN, and musk xylene were 1.3, 0.5, and 0.5 ng/g, respectively. The detection limits were 0.05 ng/g for *p,p′*-DDMU, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE, and *p,p′*-DDE, 0.2 ng/g for *o,p′*-DDT and *p,p′*-DDT, and 0.07 ng/g for HCB.

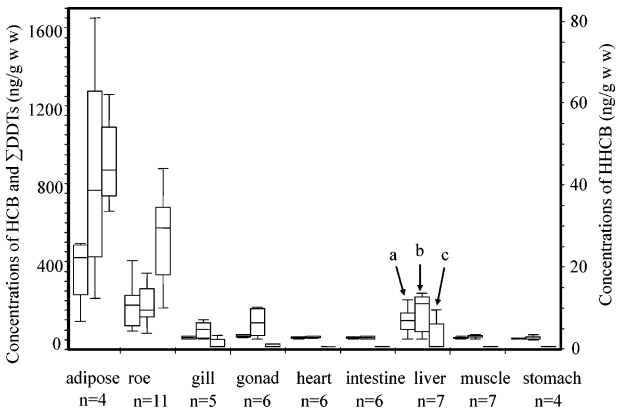

FIGURE 2. Concentrations of HCB, ∑DDTs, and HHCB (ng/g ww) in different tissues of Chinese sturgeon. Data are presented in box-and-whisker plots; 50% of the cases have values within the boxes, and the edges of the box mark the 25th and 75th percentiles. (a) HCB, (b) ∑DDTs, (c) HHCB.

tissues were relatively high (66 ± 18% for adipose, 33.7 ± 9.8% for roe, 12.2 ± 7.4% for liver). The similar tissue-distribution profile was also found for DDTs and HCB. However, when the concentrations were expressed on a lipid weight basis, the concentrations of HHCB, AHTN, and musk xylene were similar among tissues and were similar to that reported for a finless porpoise from Japan (*18*), suggesting that the tissue distributions of musks in Chinese sturgeon are controlled by the affinity to lipids in tissues. It should be noted that significant concentration differences among tissues for DDTs and HCB also disappeared when their concentrations were expressed on a lipid weight basis, implying that toxicokinetics of HHCB, AHTN, and musk xylene would be similar to those of DDTs and HCB.

Age-Related Accumulation in Roe. Previous studies indicated that age is an important factor to determine the levels of organochlorines in animals (*37*, *38*). The age trend of pollutants can provide useful information on their toxicokinetics (*39*). For mammals, many investigations of organochlorines in cetacean species found that concentrations of organochlorine generally increase with age in male whales, but decrease with age in mature female whales (*37*, *40*−*42*). Recently, Iwata et al. (*39*) studied the age trends of dioxins in liver of seals and also found significant declining trends for five isomers in female seals, and vice versa for most isomers in male seals (*39*). For fish, the increasing trends with age for DDTs, HCB, and some PCBs were found in both female and male brown trout from high mountain lake (*43*).

In our study, the concentrations of musks and organochlorines in roe represent the levels in female sturgeon, and regression analysis was conducted to investigate the relationships between the age of sturgeon and logarithmic concentrations (lipid corrected) of HHCB, AHTN, musk xylene, HCB, *p*,*p*′-DDE, and *p*,*p*′-DDD. Although no significant statistical relationships were found for HHCB, AHTN, *p*,*p*′-DDE, and *p*,*p*′-DDD (r^2 = 0.1275−0.2761, p = 0.096−0.279), and even more poor regression was found for musk xylene and HCB (r^2 = 0.0083−0.0307, p = 0.612−0.789), the lipid-corrected concentrations of HHCB, AHTN, *p*,*p*′-DDE, and *p*,*p*′-DDD were found to increase with age, as shown in Figure 3. It should be noted that the increasing trends with age for *p*,*p*′-DDE and *p*,*p*′-DDD in female Chinese sturgeons were similar to those in female brown trout and different from those reported in female mammals. Considering mass transfer of accumulated lipophilic contaminants from mother to fetus would be a factor to influence the accumulation of chemicals with age exemplified by the accumulation studies of organochlorines in whales, dioxins in seals, and butyltin compounds in dolphins (*37*, *39*, *44*), the concentrations ratios

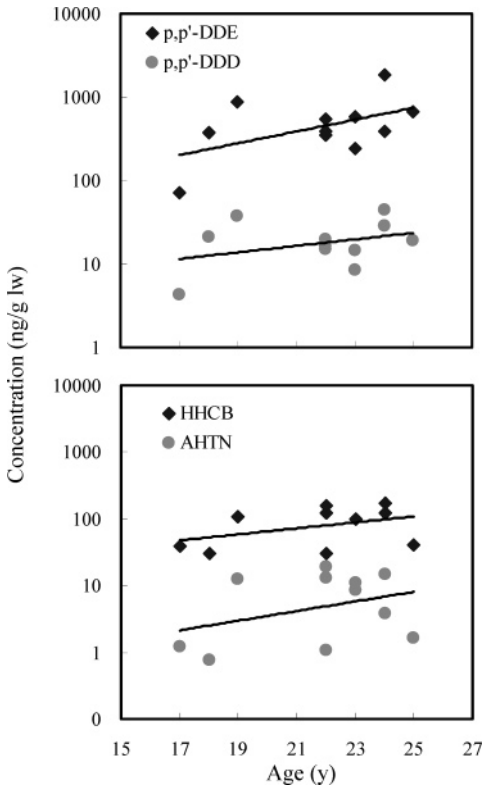

FIGURE 3. Relationships between age and concentration (ng/g lipid weight) of *p*,*p*′-DDE, *p*,*p*′-DDD, HHCB, and AHTN in roe of Chinese sturgeon. *p*,*p*′-DDE: log conc = 0.0708 × age + 1.1, r^2 = 0.2761, p = 0.096. *p*,*p*′-DDD: log conc = 0.0391 × age + 0.3959, r^2 = 0.1294, p = 0.278. HHCB: log conc = 0.0451 × age + 0.9123, r^2 = 0.1682, p = 0.207. AHTN: log conc = 0.0724 × age − 0.9002, r^2 = 0.1275, p = 0.279.

in roe to adipose based on lipid weight were calculated in two samples (A0410 and A0414). The concentration ratios in two sturgeons were 0.54 and 0.68 for HCB, and 0.24 and 0.30 for total DDTs, which were lower than those of organochlorines in whales reported in previous investigations (1.3 ± 0.6 for DDE, 1.1 ± 0.4 for total DDT) (*37*). The concentration ratios for the musks were also calculated to be 0.38 and 0.55 for HHCB, 0.39 and 0.78 for AHTN, and 0.44 and 0.58 for musk xylene, which were slightly higher than those of total DDTs and comparable to those of HCB. Such maternal transfers of these chemicals in Chinese sturgeons should decrease the body concentration of musks and DDTs. So, the increasing trends with age in Chinese sturgeons were supposed to be due to the facts that migration for reproduction occurs at intervals of more than 2 years leading to increase in the pollutant levels in Chinese sturgeon exposed in the marine environment after reproduction, in addition to the relatively low maternal transfer in fish compared with mammals.

Thus, the levels, tissue distribution, and age-related accumulation of synthetic musk fragrances and those of organochlorines in Chinese sturgeon (*A. sinensis*) were clarified for the first time, and these results provided basic data to assess the risk of these pollutants to this endangered species.

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Supporting Information Available

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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