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Synthetic Musk Residues in Biota and Water from Tama River and Tokyo Bay (Japan)

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Abstract. Musk xylene and musk ketone (synthetic musks) were detected in 100% and 80%, respectively of 74 samples, [freshwater fish (three species), marine shellfish (four species), river water, and wastewater (three sewage treatment plants)] collected from several sampling stations along the Tama River, a dam, and Tokyo Bay, during July and October, 1980 and 1981. The average concentrations of musk xylene were 53.9 ppb in the viscera of freshwater fish, 16.0 ppb in the fish muscle, and 2.7 ppb in marine shellfish; the mean levels of musk ketone were 30.5 ppb in the viscera, 7.8 ppb in the muscle, and 1.6 ppb in the shellfish. The mean levels in river water were 4.1 ppt for musk xylene and 9.9 ppt for musk ketone, but no detectable levels of musk ketone were found in freshwater fish and river water upstream of the Tama River and the dam. The highest concentrations of both compounds were observed in freshwater fish downstream, and that in water samples were found in wastewater from the sewage treatment plants, situated along the banks of the river. The results suggested that both compounds exist as bioaccumulation-type pollutants in the aquatic ecosystem.

It was reported previously that residues of the synthetic musks, musk xylene (5-*tert*-butyl-2,4,6-trinitroxylene) and musk ketone (2-acetyl-5-*tert*-butyl-4,6-dinitroxylene) have been identified in freshwater fish (*Carassius auratus langsdorfii*) collected downstream in the Tama River, which discharges into Tokyo Bay (Yamagishi *et al.* (1981a). It is well known that musks having a fragrant smell are obtained by chemical synthesis and from animal sources (the musk glands of the male musk deer and the Louisiana muskrat). Synthetic musks are of great industrial importance and include nitro and non-nitro benzenes, indans, and tetralins. Musk xylene, musk ketone, and musk ambrette are commonly called nitro-musks. The production of musk xylene and musk ketone is estimated to be 100 tons each per year in Japan (Akiyama 1980). Both compounds are used in soaps, detergents, creams, and lotions as natural musk substitutes (Okuda 1972). In addition, musk ketone is used in commercial herbicide formulations, because it is highly effective for the control of various species of weeds in greensward and nursery trees (Tomisawa and Uejii 1982). Since April 1981, the formulations have been permitted to be used as herbicides by the Japanese Ministry of Agriculture and Forestry. The acute toxicity of these compounds to mammals is relatively low; the oral LD_{50} value in rats for musk xylene and musk ketone are reported to be above > g/kg and 10 g/kg, respectively. However, a study on rabbits with musk ketone showed an increase in glutamic-pyruvic transaminase in serum on both high and low levels (Opdyke 1975). Also, musk ketone is highly toxic to aquatic organisms; LC_{50} value in 28 hr for carp is 5.1 ppm (Tomisawa and Uejii 1982).

Little is known about the fate and biological effects of musk xylene and musk ketone in the aquatic environment and, until recently there was no published data on aquatic or marine environmental pollution by musk xylene and musk ketone. (Yamagishi *et al.* 1981a). Therefore, an attempt was made to investigate fish and shellfish as indicators of aquatic environmental pollution with both musk xylene and musk ketone. The purpose of this study was to identify the routes and extent of contamination of the aquatic biota, as well as differences in

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bioaccumulation or regional differences in the concentrations of these compounds.

Material and Methods

Sampling: The sampling stations in Tama River and the dam, and Tokyo Bay are shown on the map in Figure 1. The Tama River discharges into Tokyo Bay. A total of 74 samples of freshwater fish, marine shellfish, river water, and wastewater were collected during July and October, 1980–1981 from the stations along the Tama River, the dam, and Tokyo Bay. Thirty-one samples of freshwater fish, *Zacco platypus* (pale chub), *Carassius auratus langsdorfii* (crucian carp), and *Cyprinus carpio* (carp) were caught at the 4 to 6 sampling stations in the Tama River and the dam once each year from July through September in 1980 and 1981. Nine samples of marine shellfish, *Tapes philippinarum* (short-necked clam), *Mactra chinensis* (shell), *Mytilus edulis* (mussel), and *Crassostrea gigas* (oyster) were also caught at the five sampling sites along the coast of Tokyo Bay during July 1981. Thirty-one water samples of river water and dam water were collected, using 3 L-glass bottles, from the thirteen points in Tama River and the dam, and the tributary which discharges into the Tama River during September and October, 1981. Three wastewater samples were also collected from three sewage treatment plants situated along the banks of the Tama River in September 1981. All biological samples were wrapped in hexane-washed aluminum foil and stored in a frozen state until used for analysis. The water samples were stored at 4°C.

Determination of Musk Xylene and Musk Ketone: The fish and shellfish of a given species which were taken at the same time and place were pooled as a group. Individual fish samples were dissected from the melt and separated into muscle and viscera portions. The tissue portions were pooled and homogenized in a mixer (Ultra Turrax TP 18-10). The shellfish samples were thawed and shucked. The whole meat was pooled and homogenized in the same manner as the fish samples. Musk xylene and musk ketone were extracted into acetonitrile on partitioning with *n*-hexane, and recovered from a Florisil® column after the elution of polychlorobiphenyls, DDE, BHC, and DDT (Yamagishi *et al.* 1981a). A homogenized sample of fish or shellfish (10 g) was dehydrated with anhydrous Na_2SO_4 (30 g) in a mortar, mixed with dichloromethane-hexane (1:1, 200 mL) in a 300 mL Erlenmeyer flask, and allowed to stand overnight. After filtration through a glass filter, the residue was extracted with the mixed solvent (100 mL × 2). The combined extracts were washed with water (30 mL × 2), dried with Na_2SO_4, and concentrated to 5 mL in a Kuderna-Danish evaporator. The concentrated solution was partitioned with acetonitrile (20 mL × 3). The acetonitrile layer was poured into a 2% Na_2SO_4 solution (350 mL) and, the aqueous solution was extracted with hexane (50 mL × 2). The hexane fraction was washed, dried, and evaporated to 5 mL. The extract was chromatographed on a Florisil® column. Florisil (Floridin Co. 60-100 mesh, PR grade, heated at 650°C for 18 hr, stored in a desiccator, activated at 130°C before use) was packed in a glass column (2.2 × 10 cm) with hexane. After elution with hexane (150 mL, fraction 1) and dichloromethane-hexane (1:9, 200 mL, fraction 2), musk xylene and musk ketone were eluted with acetonitrile-dichloromethane-hexane (2:10:88, 200 mL, fraction 3) together with dieldrin, CNP (a diphenyl ether herbicide), and heptachlor epoxide. Fraction 3 was concentrated to 5 mL, and subjected to gas chromatography with a ^{63}Ni electron capture (ECD-GC, Hitachi-163 type) on a 5% OV-101 column. Fraction 1 contained polychlorobiphenyls and DDE, and fraction 2 contained BHC and DDT. The water sample, after the addition of Na_2SO_4 (16 g/800 mL), was extracted with hexane (100 mL × 2), and the concentrate was chromatographed on Florisil without the partition procedure.

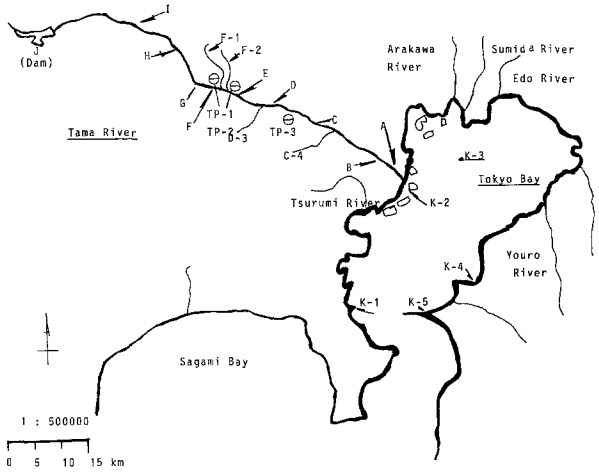

Fig. 1. Locations of sample collections

Gas chromatography was conducted under the following conditions. Columns: 5% OV-101 on 100–120 mesh, Uniport HP, at 210°C, N_2 60 mL/min; 3% OV-17 on 60–80 mesh, Gas Chrom Q, at 210°C, N_2 75 mL/min; OV-17 + OV-210; 1.5% and 1.95% on 80–100 mesh, Chromosorb W, AW DMCS, at 210°C, 75 mL/min; glass columns, 3 mm ID × 2m; injection and detector temperature 285°C.

Recoveries (average ± S.D. in five determinations) from fortified fish samples (*C. auratus langsdorfii*) at 500 ng (and 50 ng) each per 10 g level were 92.6 ± 3.1% (84.4 ± 0.9%) for musk xylene and 93.3 ± 3.3% (85.0 ± 3.0%) for musk ketone. From fortified water samples at 500 ng (and 50 ng) each per 800 mL level, the recoveries were 101.1 ± 3.1% (91.3 ± 4.0%) and 100.6 ± 2.4% (91.3 ± 1.9%), respectively. Dieldrin, heptachlor epoxide, and CNP were recovered in the range of 83.3 and 104.9% from the samples. Minimum detectable levels of musk xylene or musk ketone were 0.5 ppb in fish and 0.001 ppb in water, respectively. No interfering peaks were noted in the analysis of environmental samples.

Positive identification was based on the retention times on three different GC columns, and quantitations were based on the results with the OV-101 column. Confirmation of the presence of musk xylene and musk ketone in each pooled sample of freshwater fish, marine shellfish, river water, and wastewater was conducted by mass fragmentography with a JEOL JMS-D300 JMA 2000 Disc system instrument.

Results

Freshwater Fish and Marine Shellfish Samples: The average concentrations, the standard deviations and the ranges of musk xylene and musk ketone for all types of samples, obtained from three aquatic areas (the Tama River, the dam, and Tokyo

Table 1. Concentrations of musk xylene and musk ketone in freshwater fish and marine shellfish (ppb, wet basis) including seven species, and river water (ppt) collected from Tama River and Tokyo Bay during the summers of 1980 and 1981

Sampling site	Sample	Sample no.	Average, Range	Musk xylene	Musk ketone
1980					
Dam and river	Freshwater Fish —Muscle—	7	Mean	13.1	3.4
			Std. dev.	9.7	5.4
			Range	1.1–32	nd–16
1981					
Dam and river	Freshwater Fish —Muscle—	12	Mean	17.7	10.5
			Std. dev.	12.4	11.8
			Range	1.5–41	nd–27
	—Viscera—	12	Mean	53.9	30.5
			Std. dev.	44.9	26.3
			Range	1.4–140	nd–70
	River water[1]	18	Mean	4.1	9.9
			Std. dev.	1.9	5.9
			Range	1.7–7.9	nd–19
	River water[2]	13	Mean	15.0	11.8
			Std. dev.	3.1	5.3
			Range	10–23	7–28
	Wastewater from sewage treatment plants	3	Mean	32.0	270
			Std. dev.	5.0	110
			Range	25–36	140–410
Tokyo Bay	Marine shellfish	9	Mean	2.7	1.6
			Std. dev.	1.1	0.6
			Range	1.7–5.3	0.9–2.6

[1] The flowing water in the Tama River

[2] The flowing water in the tributaries which is discharged into the Tama River

Bay), are summarized in Table 1. Musk xylene and musk ketone were detected in 100% and 80% of the 74 analyzed samples, respectively. In 1981, for freshwater fish including three species of *Z. platypus, C. auratus langsdorfii,* and *C. carpio,* the average concentration of musk xylene residues in muscle was 17.7 ppb with a range of 1.5 to 41 ppb. Musk ketone levels in the fish muscle ranged from "not detectable" to 27 ppb with a mean of 10.5 ppb. Both musk xylene and musk ketone residues in marine shellfish (four species of *T. philippi narum, M. chinensis, M. edulis,* and *C. gigas*) ranged between 1.7 to 5.3 ppb, but no trend was apparent. The residual levels of musk xylene and musk ketone in freshwater fish samples obtained from the downstream areas in the Tama River, generally, were higher than in those of the upstream and the dam areas. The highest concentrations of both residues were found in freshwater fish from the downstream areas in the Tama River in both years 1980 and 1981 (Table 2). The concentrations of the residues found in the fish were higher in the viscera than in the muscle tissues. Also, musk ketone levels in the tissue portions were 1/2 to 1/3 as low as those of musk xylene. The differences in these residual levels were also found in marine shellfish, but each concentration was low compared to the levels found in the fish (Tables 2, 3, 4).

Water Samples: The concentrations of musk xylene and musk ketone in river water, dam water, and wastewater from the sewage treatment plants, which are situated along the banks of the Tama River, are given in Tables 5 through 7. Musk xylene was found in all water samples. In the river water from upstream to downstream areas in the river, musk xylene levels were constant with a range of 2 to 7.9 ppt. However, no musk ketone was detected in the dam and the upstream areas in the river. The highest concentration (19 ppt) of musk ketone in the river water was found in the middlestream areas (Table 6). For a 24-hr sampling period of river water, both taken at F-1 point of a tributary and the main stream (F point) of the Tama River, the concentrations are given in Tables 7 and 8. The concentration changes of musk xylene and musk ketone in the flowing water of the tributary, were similar to that of the main stream; no trends with times of sampling were apparent in the concentrations. However, the results of the times analyses showed that the concentrations of musk xylene in the tributary were at significantly higher levels than those of

Table 2. Concentrations (ppb, wet basis) of musk xylene and musk ketone residues in freshwater fish collected from the Tama River during the summers of 1980 and 1981

Sampling site[b]	Distance (km) from estuary	Species	Range of body weight (g)	Musk xylene[c]		Musk ketone	
				Muscle	Viscera	Muscle	Viscera
1980							
C	20.8	*C. auratus langsdorfii*	24–36	32		16	
F	43.7	*C. auratus langsdorfii*	28–76	15		3.9	
G	46.4	*Z. platypus*	11–24	13		3.7	
		C. auratus langsdorfii	57–95	11		nd[a]	
		Z. platypus	10–16	18		nd	
J	88.0	*C. auratus langsdorfii*	62–90	1.7		nd	
		C. carpio	100–144	1.1		nd	
1981							
B	13.3	*Z. platypus*	9–12	41	140	27	70
C	20.8	*Z. platypus*	10–11	28	69	28	30
		C. auratus langsdorfii	130–160	10	36	7.5	51
		C. carpio	121–220	6.1	58	2.0	35
E	40.4	*Z. platypus*	11–18	11	30	8.4	17
		C. auratus langsdorfii	55–73	6.1	19	1.5	39
F	43.7	*Z. platypus*	11–13	32	140	22	75
		C. auratus langsdorfii	25–37	31	76	29	49
G	46.4	*Z. platypus*	22–31	21	54	nd	nd
		C. auratus langsdorfii	53–104	20	20	nd	nd
J	88.0	*Z. platypus*	28–34	1.5	3.3	nd	nd
		C. auratus langsdorfii	39–46	5.1	1.4	nd	nd

[a] nd = not detectable
[b] The sites are arranged from downstream to upstream and the dam
[c] Data represent mean of triplicate analyses of a pooled sample of five fish

Table 3. Concentrations (ppb, wet basis) of musk xylene and musk ketone residues in marine shellfish (*T. philipinarum*) collected from Tokyo Bay, July 1981[a]

Sampling site[b]	Musk xylene	Musk ketone
K-1	2.0	1.0
K-2	2.3	2.3
K-3	1.7	1.1
K-4	2.2	1.5
K-5	2.2	2.0
Mean	2.1	1.6
Std. Dev.	0.2	0.5

[a] Data are mean of triplicate analyses of a pooled sample from sixty shellfish
[b] The sites are shown on the map in Figure 1

Table 4. Concentrations (ppb, wet basis) of musk xylene and musk ketone residues marine shellfish of four species collected from a sampling station (K-3)[b] in Tokyo Bay, July 1981[a]

Species	Musk xylene	Musk ketone
T. philipinarum	2.0	0.9
M. chinesis	2.2	1.9
M. edulis	4.0	2.0
C. gigas	5.3	2.6
Mean	3.4	1.7
Std. Dev.	1.4	0.7

[a] Data are mean of triplicate analyses of a pooled sample from sixty shellfish
[b] The site is shown on the map in Figure 1

Table 5. Concentrations (ppt) of musk xylene and musk ketone in flowing water collected from nine sampling stations in the Tama River and the dam, September 23–26, 1981

Sampling site[b]	Distance (km) from estuary	Musk xylene	Musk ketone
Estuary	0	—	—
A	6.0	—	—
B	13.3	6.0	7.5
C	20.8	5.6	7.8
D	32.9	3.4	13
E	40.4	3.9	19
F	43.7	5.6	9.1
G	46.4	7.2	9.5
H	54.2	7.9	nd[a]
I	71.1	5.0	nd
J(Dam)	88.0	2.0	nd

[a] nd = not detectable, <0.05 ppt
[b] The sites are shown on the map in Figure 1

Table 6. Concentrations (ppt) of musk xylene and musk ketone in flowing water collected from four tributaries which discharge into the Tama River without any treatment

Sample site[a]	Musk xylene	Musk ketone
F-1	16	11
F-2	17	16
D-3	14	12
C-4	23	28
Mean	17.5	16.8
Std. Dev.	3.4	6.8

[a] The sites are shown on the map in Figure 1

the main stream. The levels in water samples from the sewage treatment plants were high with a mean of 32 ppt for musk xylene and 270 ppt for musk ketone, compared to the above three water samples (Table 9).

Discussion

Until recently, environmental pollution with musk xylene and musk ketone was not apparent, because of analytical difficulties due to masking by other ubiquitous pollutants such as polychlorobiphenyls and organochlorine pesticides. Moreover, it is commonly considered that musk xylene and musk ketone would not be accumulated in the biota when released into the environment, because aromatic nitro compounds are known to be degraded by microbial metabolism or by a nonbiological pathway in the soil (Williams 1957; Kuwatsuka 1977). The present investigation, revealed that musk xylene and musk ketone were commonly found in freshwater fish and marine shellfish from the Tama River and Tokyo Bay, and also in the river water. The results indicate that these compounds exist as

Table 7. Changes during 24 hr in concentrations (ppt) of musk xylene and musk ketone in flowing water collected from sampling station (F)[a] in the Tama River, October 13–14, 1981

Sampling time hr	Musk xylene	Musk ketone
0	5.5	21
6	1.7	13
8	4.2	9.9
10	3.0	9.5
12	2.7	19
14	3.1	9.3
16	2.7	11
18	1.7	11
20	2.0	9.3
Mean	2.9	12.6
Std. Dev.	1.2	4.2

[a] The site is shown on the map in Figure 1

Table 8. Changes during 24 hr in concentrations (ppt) of musk xylene and musk ketone in flowing water of the tributary (F-1)[a] which is discharged into the Tama River, September 29–30, 1981

Sampling time hr	Musk xylene	Musk ketone
0	15	15
6	12	9
8	13	10
10	17	8
12	16	11
14	15	10
16	12	7
18	15	10
20	10	7
Mean	13.9	9.7
Std. Dev.	2.1	2.3

[a] The site is shown on the map in Figure 1

Table 9. Concentrations (ppt) of musk xylene and musk ketone in wastewater collected from three treatment plants along the Tama River

Sampling site[b]	Musk xylene	Musk ketone
TP[a]-1	25	140
TP-2	35	260
TP-3	36	410
Mean	32.0	270
Std. Dev.	5.0	110

[a] The sewage treatment plants
[b] The sites are shown on the map in Figure 1

bioaccumulation-type pollutants in the aquatic or marine environmental ecosystem. It was observed that the bioaccumulation ratio of musk xylene and musk ketone found in freshwater fish was different. A mean bioaccumulation ratio found in muscle of the fish was calculated to be 4.1×10^3 for musk xylene and 1.1×10^3 for musk ketone. This difference in accumulation by fish may be explained as follows: Metabolic activity of the fish and microbial degradation in the river water may have influenced the difference observed in bioaccumulation ratios, and may be related with the moiety of the molecules, especially the number of nitro groups, although this is not clear at this time. In either case, it is clear that both musk xylene and musk ketone are accumulated by fish and shellfish. This may also give a clue to the fact that a chemical substance with an aromatic nitro compound, for example, a diphenyl ether herbicide (CNP), is accumulated by fish and shellfish in Tokyo Bay and its surrounding river after application in the agricultural ground of a rice paddy field (Yamagishi *et al.* 1978; Yamagishi and Akiyama 1981).

As for the difference of the residual levels between muscle and viscera found in freshwater fish, this may be due to differences in their lipid contents. Also, data from marine shellfish showed that the levels in *C. gigas* (oyster) and *M. edulis* (blue mussel), which have high lipid contents, were higher than those in *T. philippinarum* (short-necked clam) and *M. chinesis* (shell), which have relatively low lipid contents (Miyazaki *et al.* 1980). Thus, these results indicate that fatty tissues accumulate both musk xylene and musk ketone, selectively.

It is apparent that downstream areas in the Tama River have been severely contaminated with both musk xylene and musk ketone, as compared with the upstream, the dam or Tokyo Bay areas (Table 1 and Figure 2). The results indicate that the concentrations of both residues in the freshwater fish probably reflects the contamination in the estuarial areas, which are accumulation sites for both compounds, at least in a short term (Yamagishi *et al.* 1981b). Cutshall *et al.* (1981) and Simpson *et al.* (1976) reported that contaminants such as organic chemicals, which are dissolved or associated with fine-grained sediment, are transported downstream and deposited in estuaries where river flocculation occurs. Whereas in all water samples, the highest concentrations were found in wastewater from the sewage treatment plants, which are situated along the banks of the downstream in the river. Also, data from river water samples showed that the higher concentrations were found in midstream. Therefore, it was reasonable to assume that the major

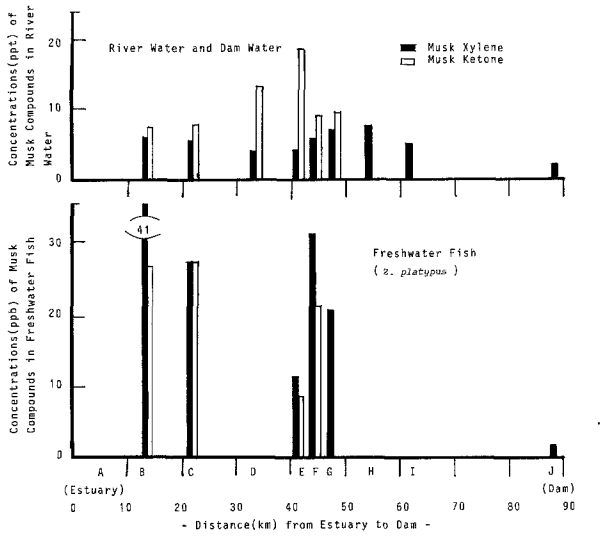

Fig. 2. Changes in concentrations of musk xylene and musk ketone in freshwater fish (*Z. platypus*), river water and dam water of the Tama River

factor of the contaminations by both musk xylene and musk ketone was probably due to the domestic wastewater from the cities. In fact, the cities along the river have increased in population and in the volume of wastewater. With or without treatment, the major part of the wastewater from the cities is discharged into the main stream of the Tama River. However, it was observed that the residual levels in marine shellfish from Tokyo Bay were lower than in the freshwater fish. The low levels in the shellfish may reflect the concentrations in seawater, which are diluted and mixed with the river water. The rainfall and the dry season may also affect the residue concentrations in the shellfish, because the Tama River which is a large river flowing into the Bay includes 60% of the total wastewater from houses and industries in the Tokyo area.

It is emphasized that pollution may be occurring with musk xylene and musk ketone in aquatic biota and it is necessary to continue surveys to determine whether these substances will be concentrated in the aquatic environment and what their effects may be.

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