Detection of Pesticides Unregistered in Japan, Toxaphene and Mirex, in the Cetaceans from Japanese Coastal Waters

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Introduction

Toxaphene and mirex are the members of persistent organic pollutants (POPs) included in the International Treaty, the Stockholm Convention. POPs are characteristically transferred across borders and are accumulated in wildlife. Consequently, their global distribution and biological impacts are of great concern. However, there is very little information on contamination by toxaphene and mirex in the Asia-Pacific region. In Japan, scientific research focused on these two pesticides has not been conducted so far since they have never been registered there.

In this study, toxaphene and mirex were determined in the blubber of cetaceans collected from various regions with a focus on Asian countries to evaluate the effects of their long-range atmospheric transport from regions where they were extensively used.

Methods and Materials

Samples: Forty specimens of cetaceans including Dall's porpoise (*Phocoenoides dalli*), finless porpoise (*Neophocaena phocaenoides*), Stejneger's beaked whale (*Mesoplodon stejnegeri*), Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), harbour porpoise (*Phocoena phocoena*), spinner dolphin (*Stenella longirostris*) and Indo-Pacific humpback dolphin (*Sousa chinensis*) collected from the Asian coastal waters such as Japan, Hong Kong, India and the Philippines during 1990 and 2001 were used in this study. For comparison, 15 specimens including franciscana (*Pontoporia blainvillei*) and estuarine dolphin (*Sotalia guianensis*) accidentally caught in Brazil and harbour porpoise collected from

Black Sea, Turkey during 1993 and 1999 were also included in this study. Blubber samples stored at -20ºC were employed for chemical analysis.

Sample Preparation: Extraction and clean-up procedures for toxaphene (Parlar-26, -32, -50, -62 and -69) and mirex followed the method reported by Fowler[1] and Chan[2]. Briefly, blubber samples (1-2 g) spiked with surrogate solutions ($^{13}C_{10}$-*trans*-chlordane for toxaphene and $^{13}C_{12}$-PCB101 for mirex) were homogenized and then extracted with 1:2 acetone/hexane. After centrifugation and partitioning with hexane-washed water, the extract was added to a gel permeation chromatography column (GPC; Bio-Beads S-X3, Bio-Rad Laboratories) for lipid removal by equivalent mixture of dichloromethane in hexane. The fraction containing toxaphene and mirex was concentrated and passed through a Florisil column with 5% dichloromethane in hexane for further clean-up. As internal standards for toxaphene and mirex, $^{13}C_{12}$-PCB170 and $^{13}C_6$-hexachlorobenzene were added to final solution prior to GC-MS analysis, respectively. Lipid contents were determined gravimetrically.

GC-MS: Quantification of toxaphene and mirex was performed using a GC (Agilent 6980N) equipped with MSD (Agilent 5973N) using NCI-SIM and EI-SIM mode, respectively. GC columns used for quantification were DB-5MS fused silica capillary (J&W Scientific Inc.) having 0.25 mm i.d. x 60 m x 0.25 µm film thickness for toxaphene, and HT-8 capillary column (SGE Japan Inc.) having 0.22 mm i.d. x 50 m x 0.25 µm film thickness for mirex. The concentration was expressed on a lipid weight basis.

Results and Discussion

Toxaphene and mirex were detected in almost all the blubber samples analyzed in this study with concentrations ranging from <10 to 6700 ng/g lipid wt and 2.0 to 2800 ng/g lipid wt, respectively (Table 1). To our knowledge, this is the first comprehensive study to detect these pesticides in the biological specimens from Asia-Pacific waters including Japan where these chemicals have never been registered. This study highlighted widespread contamination by toxaphene and mirex in the aquatic ecosystem not only in the environment where they have been used but also the areas where they have never been used.

Table 1. Toxaphene and mirex concentrations (mean and range in parentheses, ng/g on lipid weight basis) in the blubber of cetaceans collected from Asia, Brazil and Black Sea

Location	Species	Year	*n*	Lipid (%)	P26	P32	P50	P62	P69	Total Toxaphene	Mirex
Japan	Dall's porpoise (*truei* -type)	1998-1999	6	79	1500	<10	1600	230	<1.0	3300	66
				(64-85)	(370-3900)		(530-2500)	(100-310)		(1000-6700)	(5.0-170)
	Dall's porpoise (*dalli* -type)	2000	5	86	1500	<10	1500	170	<1.0	3100	65
				(80-99)	(820-2100)		(700-2300)	(72-280)		(1600-4700)	(34-94)
	Finless porpoise	1999-2000	4	53	180	<10	120	28	<1.0	320	10
				(42-63)	(65-340)		(70-150)	(7.0-56)		(140-550)	(2.0-24)
	Stejneger's beaked whale	2000-2001	2	78	260	<10	61	14	<1.0	330	51
				(63-92)	(250-260)		(56-66)	(13-14)		(320-340)	(33-69)
	Pacific white-sided dolphin	1999	5	69	820	<10	1400	150	<1.0	2400	36
				(46-84)	(83-1700)		(150-3100)	(30-290)		(270-5100)	(4.0-77)
	Harbour porpoise	1999	3	87	230	<10	320	41	<1.0	590	8.0
					(67-360)		(100-440)	(21-55)		(190-850)	(4.0-12)
Hong Kong	Finless porpoise	2000	5	47	18	<10	24	<1.0	<1.0	42	150
				(32-65)	(8-32)		(8.0-37)			(16-69)	(54-300)
	Indo-Pacific humpback dolphin	2000-2001	5	26	88	<10	81	<1.0	<1.0	170	1100
				(17-34)	(<1.0-270)		(<1.0-250)			(<10-520)	(79-2800)
The Philippines	Spinner dolphin	1996	2	51	410	<10	790	120	<1.0	1300	40
				(49-52)	(320-490)		(590-990)	(86-160)		(1000-1600)	(39-40)
India	Spinner dolphin	1990-1992	3	66	410	<10	780	81	<1.0	1300	15
				(53-73)	(150-810)		(210-1600)	(32-130)		(390-2500)	(7.0-24)
Brazil	Estuarine dolphin	1998-1999	5	69	68	<10	96	5.0	<1.0	170	290
				(58-79)	(38-150)		(60-210)	(<1.0-10)		(110-370)	(110-730)
	Franciscana	1998-1999	5	80	7.0	<10	13	<1.0	<1.0	20	64
				(73-86)	(4.0-10)		(8.0-17)			(12-26)	(34-86)
Black Sea	Harbour porpoise	1993	5	88	730	<10	970	56	<1.0	1700	10
				(82-90)	(590-960)		(700-1400)	(40-82)		(1300-2400)	(7.0-13)

Toxaphene. Figure 1 shows the geographical distribution of toxaphene concentrations in mature male samples. In general, toxaphene levels were higher in the specimens from Japan followed by Black Sea, the Philippines and India. Although toxaphene was not registered in Japan, the highest residue level was found in Dall's porpoise collected from northern Japan in 1999. The levels in the animals near Hong Kong and Brazil were much lower. Residue levels observed in this study were in the range found in cetaceans from Canada, one of the areas considered to be most polluted by these compounds[3,4,5,6]. Among the five congeners, Parlar-26, -50 and -62 were detected in most of the samples analyzed (Table 1). On the other hand, the other two congeners, Parlar-32 and -69, were below the limit of detection at 10 and 1.0 ng/g lipid wt, respectively in all the samples. Accumulation pattern observed in this study was similar to the findings in cetacean samples from other regions[1,2].

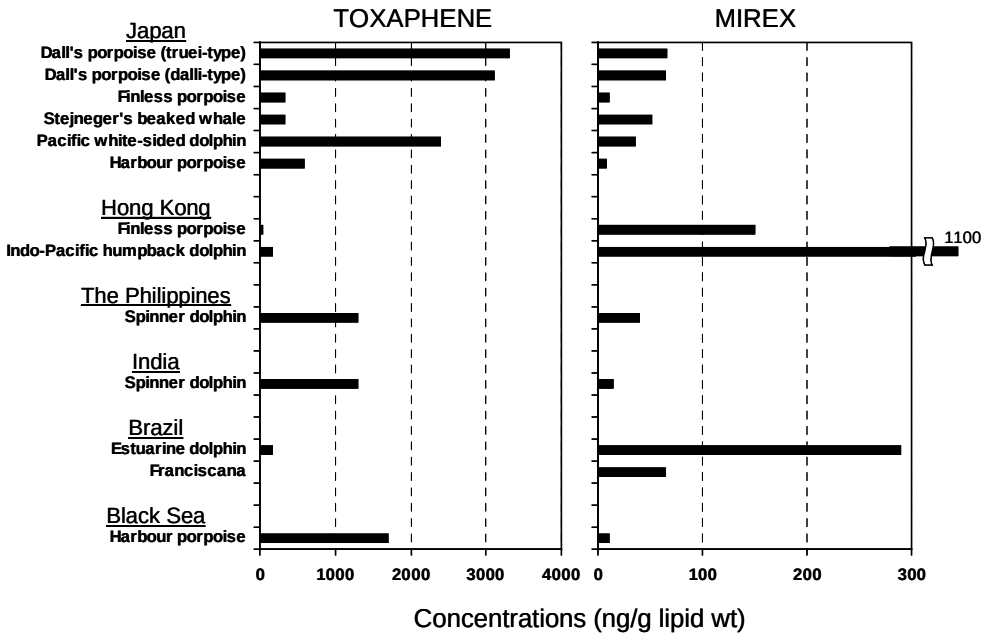

Figure 1. Geographical distribution of toxaphene and mirex concentrations in the blubber of cetaceans

In the cetaceans stranded in Japan, concentrations in Dall's porpoise (mean: 3700 ng/g lipid wt) and Pacific white-sided dolphin (mean: 3100 ng/g lipid wt), which are offshore species migrating to the North Pacific, Sea of Okhotsk and Japan Sea,

were much higher than those in Japanese coastal species including finless porpoise (mean: 250 ng/g lipid wt) and harbour porpoise (mean: 740 ng/g lipid wt). This result may confirm that this pesticide was not used in Japan in the past. It is noteworthy that, although toxaphene is not registered in Japan, offshore species around Japan showed higher levels than harbour porpoise (mean: 1800 ng/g lipid wt) collected from Black Sea, Turkey where the surrounding former Soviet countries like Ukraine are the regions of higher toxaphene consumption[7]. These results indicate that considerable amount of toxaphene has been transported to the environment around Japan via atmosphere, a phenomenon common for POPs. Toxaphene has been reported to have comparable transportability to HCHs and PCBs[8]. Since toxaphene has been used in India and Cambodia in the past[9], South Asian countries might be one of the possible pollution sources. Atmospheric transport from the US also should be taken into account. Due to high consumption of toxaphene in the US, up to 40% of the world demand[10], total residual levels in the agricultural soil there are estimated to be much higher than in the Asian countries[11]. Since long-range atmospheric transport of toxaphene originating from the US has been reported to affect the environment of Canada[10], it may be plausible to consider that their impact might extend up to the North Pacific where Dall's porpoises migrate. Additionally, atmospheric transport from the former Soviet Union may not be ignored with regard to the input to the Japan Sea. The amount of use of toxaphene as a pesticide there was the second highest in the world[9]. Relatively high accumulation levels of toxaphene in the cetaceans from India and the Philippines may reflect its usage there and in surrounding nations.

Mirex. Mirex was also detected in all the five species from Japanese waters with concentrations from 2.0 to 170 ng/g lipid wt, although it is an unregistered pesticide in Japan (Table 1). The geographical distribution of mirex is apparently different from toxaphene (Figure 1). The highest mirex concentrations were detected in the cetaceans from Hong Kong followed by the specimens from Brazil, Japan (offshore species), India and the Philippines in order. Japanese coastal species and harbour porpoise from Black Sea showed the lowest residue levels. These levels were lower than the previously reported values in cetaceans from US [12,13]. Since mirex had the lowest transportability among POPs[8], high residue levels in Hong Kong and Brazilian cetaceans might be reflecting their own usage and/or direct input from surrounding nations. Concentrations of mirex detected in the offshore species from Japanese waters were higher than those in the coastal species, suggesting its atmospheric transport potency to some extent.

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