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Contamination Levels and Specific Accumulation of Persistent Organochlorines in Caspian Seal (*Phoca caspica*) from the Caspian Sea, Russia

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Abstract. Persistent organochlorines, such as polychlorinated biphenyls (PCBs) including coplanar congeners, DDTs, HCHs, chlordanes (CHLs), and HCB, were determined in the blubber of Caspian seals (*Phoca caspica*) and their fish diet (*Rutilus* sp.) collected in 1993 from the northern Caspian Sea, Russia. Notable concentrations of DDTs and HCHs were found at mean values of 19 and 1.3 μg/g on wet-weight basis in adult male seals, respectively. PCB pollution in Caspian seals was not so considerable compared with those of seals that suffered mass mortality. Less gender difference of organochlorine residue levels in adult animals implies less excretion of organochlorines from the body of adult females through lactation and gestation, probably due to the higher rate of pregnancy failure. Immature seals had a wide range of organochlorine concentrations, which decreased as body length increased, suggesting dilution. Caspian seals can be considered to have higher degradation capacity for coplanar PCBs. Mean TEQs (2,3,7,8-TCDD toxic equivalents) for non-, mono- and di-*ortho* coplanar PCBs was 51 pg/g on wet-weight basis, which was lower than those in seals that have suffered mass mortality, but comparable to those found in Arctic seals.

Persistent organochlorines, such as PCBs (polychlorinated biphenyls), DDTs (dichlrodiphenyltrichloroethane and its metabolites), HCHs (hexachlorocyclohexane isomers), CHLs (chlordane related compounds), and HCB (hexachlorobenzene), have been of a great concern due to their toxic effects on humans and wildlife. These contaminants have been detected in a wide range of environmental media and biota not only in developed nations but also in some developing countries, particularly in Asia and Oceania (*e.g.,* Iwata *et al.* 1993, 1995; Kannan *et al.* 1997; Prudente *et al.* 1997). However, environmental contamination by organochlorines in the former USSR and Middle East countries have been scarcely surveyed.

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The Caspian Sea, the biggest landlocked body of salt water, borders the Russia, Kazakhstan, Azerbaijan, Turkmenistan, and Iran. Environmental pollution due to fuel leakage from offshore oil wells and waste water effluents from industrial and human activities from the river basin and coastal urban areas of the Caspian Sea has been reported recently (Karpinsky 1992). Contamination by organochlorines has also been reported as one of the significant environmental problems in this area (Ballschmiter *et al.* 1983; Hooper *et al.* 1997). In this context, Caspian seal (*Phoca caspica*) has been noted as one of the contaminated biota by organochlorines (Andersson *et al.* 1988; Vetter *et al.* 1995). This seal is a *phocine* relict inhabiting only the Caspian Sea and absolutely isolated from other seals in the other marine environments. As the top predator in this ecosystem and a migratory habitat that extends from northern Caspian Sea in winter to southern waters in summer, the Caspian seal is a good bioindicator for understanding organochlorine contamination status in the Caspian Sea.

Marine mammals have been known to be highly contaminated by organochlorines and have been greatly affected by these toxic compounds. In fact, occurrence of reproductive impairment, immune suppression, cancer, and so on in some species of marine mammals were linked to accumulation of elevated levels of organochlorines (Helle *et al.* 1976; de Swart 1995; Colborn and Smolen 1996). Although the causative factor is still unclear, the declining population of Caspian seals has been suspected partly due to low pregnancy rates (Krylow 1990), and an unusually large number of corpses (several thousands) was found washed off the middle coast of the Caspian Sea in 1997 (Eybatov 1997). Notwithstanding these concerns regarding Caspian seals, very few studies have been conducted on the toxic contaminant residues in their bodies.

The present study aims at elucidating the status of contamination, specific accumulation with age and sex, and metabolic capacity of organochlorines in Caspian seals. In this study, chemical analysis and assessment of toxic potential of coplanar PCBs are also examined.

Materials and Methods

Samples

The biometry of Caspian seals and their fish diet (roach; *Rutilus* sp.) used in this study are shown in Table 1. Sixteen male and 28 female Caspian seals and three diet fishes were collected at Pearl Island (N 45°00′, E 48°17′), northern Caspian Sea, Russia, in November 1993. The blubber samples of seals and the whole body homogenate of fish were employed for chemical analysis. Age of the seal was determined by counting the growth layer groups in the dentine following the procedure of Kasuya *et al.* (1976). Popov (1982) reported that sexual maturity of male and female animals is at 6–7 and 7–8 years, respectively. Roach is the common diet of Caspian seal in northern Caspian Sea (Popov 1982).

Chemical Analysis

Chemical analysis of PCBs including non-*ortho* coplanar congeners followed the methods of Wakimoto *et al.* (1971) and Tanabe *et al.* (1987b). About 5 g of seal blubber and 20 g of the fish homogenate were refluxed with 250 ml of 1 N KOH-ethanol solution for 1 h. PCBs in the extract were transferred to 100 ml of hexane in a separatory funnel. Hexane layer was concentrated and passed through activated silica-gel (Wako-gel S-1: Wako Pure Chemical Industries Ltd., Osaka, Japan) packed in a glass column. PCBs were eluted with 200 ml hexane. After concentration up to 6 ml by a KD concentrator, 25-ml aliquot of this solution was used for the analysis of non-*ortho* coplanar PCBs (IUPAC 77, 126, and 169). The remaining 1 ml was cleaned up with 5% fuming sulfuric acid in concentrated sulfuric acid and then washed with water. This extract was used for analyzing PCB congeners other than non-*ortho* coplanar PCBs.

For non-*ortho* coplanar PCBs, 5 ml of extract through 125 mg of activated carbon column to separate non-*ortho* coplanar PCBs from the other PCB congeners. First fraction eluted with 100 ml of 20% dichloromethane in hexane was discarded. The second fraction eluted with 100 ml of 50% of ethylacetate in benzene was collected for determining non-*ortho* coplanar congeners. This fraction was concentrated nearly to dryness using a rotary evaporator and then reconstituted with hexane. The extract was cleaned up with 5% fuming sulfuric acid in concentrated sulfuric acid and then washed with water.

Identification and quantification of PCB congeners was performed using a HRGC-MSD (Hewlett-Packard, DE, 5980 series II and 5792 mass selective detector) in selective ion monitoring (SIM) mode. Separation was accomplished with capillary column coated with DB-1701 and DB-1 (J&W Scientific, CA, 0.25 mm ID and 30 m length) for non-*ortho* coplanar PCBs and *ortho*-substituted congeners, respectively. An equivalent mixture of Kanechlor® 300, 400, 500, and 600 (Kanegafuchi, Japan) was used as an external standard. Cluster ions were monitored at m/z 256, 290, 324, 358, 392, and 428 to determine the concentration of tri-, tetra-, penta-, hexa-, hepta-, and octachlorobiphenyls, respectively.

Besides PCBs, other organochlorines, such as DDTs, HCHs, CHLs, and HCB, were analyzed following the method of Tanabe *et al.* (1994). Four grams of seal blubber and 10 g of fish homogenate were ground with anhydrous sodium sulfate and extracted in a Soxhlet apparatus with a mixture of diethyl ether and hexane. The extract was added to a 20 g florisil packed glass column and then dried by passing nitrogen gas. Organochlorines adsorbed onto florisil were eluted with 150 ml of 20% water in acetonitrile to a separatory funnel containing hexane and water. After partitioning, a hexane layer was concentrated and then passed through 8 g of activated florisil for separation. The first fraction eluted with hexane contained PCBs, *p,p′*-DDE, *trans*-nonachlor, and HCB; the second fraction eluted with 20% dichloromethane in hexane contained other organochlorines.

The quantification of organochlorine residues was performed using a gas chromatograph (Hewlett-Packard 5890 series II) equipped with ECD (electron capture detector) and a moving needle-type injection port (splitless and solvent cut mode, Shimazu Co. Ltd., Kyoto, Japan). The GC column used was a fused silica capillary (DB-1; J&W Scientific, 0.25mm ID and 30 m length). The concentration of individual organochlorines was quantified from the peak area on the samples to that of the corresponding external standard.

Results and Discussion

Status of Contamination

Organochlorine contaminants were detected in all the samples of Caspian seal and fish examined (Table 1). DDTs in seals were the dominant contaminants with concentrations ranging from 5.6 to 88 μg/g (wet weight), followed by PCBs (2.2–23 μg/g), HCHs (0.13–2.0 μg/g), CHLs (63–500 ng/g), and HCB (2.4–77 ng/g). Fish samples contained PCBs at the highest concentration of 33 ng/g, followed by DDTs at half of the concentrations of PCBs.

Relatively higher concentrations of DDTs and HCHs were found in male Caspian seals, compared with those in other species of seals from various regions of the world (Table 2). Mean DDTs concentrations were 14 μg/g and 19 μg/g (wet weight) in immature and mature male Caspian seals, respectively. These observed concentrations in Caspian seals were comparable or higher than those in seals that suffered from mass mortality in Europe, but several times lower than the concentrations found in seals from Lake Baikal and the Baltic Sea. In the former USSR, technical DDT application was regulated for agricultural purpose in 1970, and its production was terminated in the 1980s (Barrie *et al.* 1992). In spite of earlier restriction on DDT production and usage, relatively high concentrations of DDTs have been detected in fish, aquatic mammals, and human from the former USSR (Nakata *et al.* 1995, 1998; Tanabe *et al.* 1997; Hooper *et al.* 1997; AMAP 1998). These observations may suggest continuous usage of DDT and/or its slower degradation rate in such colder regions.

Male Caspian seals retained notably higher concentrations of HCHs (mean concentrations in immature and mature seals were 1.0 and 1.3 μg/g wet weight, respectively) compared to those in other seals (Table 2). According to Barrie *et al.* (1992), technical HCH mixture was used extensively in the former USSR until its restriction in 1986, but lindane (γ-HCH) is still being used in recent years. Li *et al.* (1996) estimated that the usage of this insecticide in 1980 was concentrated in Azerbaijan, which borders the western coast of Caspian Sea. In Kazakhstan, which borders the northeastern coast of Caspian Sea, considerably higher concentrations of β-HCH were recorded in human breast milk, at concentrations comparable to those found in China and India in the early 1980s (Hooper *et al.* 1997). These observations suggest notable contamination of HCHs in Caspian seals from extensive usage of this insecticide around the Caspian Sea in the past and present.

Mean concentrations of PCBs in immature and mature male Caspian seals were 7.4 μg/g and 9.7 μg/g wet weight, respectively (Table 2). Concentrations of PCBs were lower than those in seals inhabiting industrial areas, but higher than those in the

Table 1. Biometry and organochlorine concentrations in the blubber of Caspian seals and their fish diet from Northern Caspian Sea

Sample No.	Sex*	Age (years)	BL** (cm)	BW** (kg)	Lipid (%)	Concentrations (μg/g wet weight) PCBs	DDTs	HCHs	CHLs	HCB
Caspian seal (*Phoca caspica*)										
CS01	m	0.5	77.0	14.0	80	8.7	22	1.0	0.29	0.0092
CS02	m	0.5	94.5	19.6	87	5.0	11	1.2	0.20	0.0069
CS03	m	0.5	81.0	15.0	84	7.2	20	1.2	0.29	0.019
CS04	m	0.5	85.0	14.2	84	4.4	11	0.90	0.14	0.014
CS05	m	0.5	80.5	11.6	75	9.5	11	1.1	0.21	0.010
CS06	m	0.5	86.5	19.2	84	6.5	14	1.0	0.22	0.014
CS07	m	4.5	104.0	29.5	89	3.2	11	0.90	0.23	0.010
CS08	m	4.5	111.0	38.6	86	3.8	7.2	0.56	0.15	0.0091
CS09	m	4.5	97.0	22.3	89	4.0	11	1.0	0.17	0.013
CS10	m	4.5	99.5	23.0	90	6.0	17	1.3	0.22	0.032
CS11	m	6.5	110.0	32.5	71	4.2	8.6	1.0	0.15	0.014
CS12	m	8.5	111.0	49.0	89	7.9	13	1.3	0.25	0.020
CS13	m	19.5	115.0	38.0	88	14	25	1.7	0.31	0.0030
CS14	m	21.5	122.0	58.0	89	9.8	21	1.9	0.26	0.0069
CS15	m	22.5	117.0	58.5	91	7.7	17	0.91	0.24	0.010
CS16	m	un	116.0	46.6	86	15	30	0.86	0.34	0.014
CS17	f	0.5	85.0	16.2	85	4.1	15	0.93	0.17	0.013
CS18	f	0.5	72.0	9.7	76	11	45	1.7	0.46	0.016
CS19	f	0.5	82.0	18.0	87	4.5	27	1.2	0.32	0.0062
CS20	f	1.5	90.0	18.9	87	3.9	15	0.83	0.15	0.027
CS21	f	2.5	86.0	15.0	94	7.0	23	1.4	0.35	0.012
CS22	f	2.5	91.0	16.7	87	4.5	20	0.88	0.19	0.0049
CS23	f	2.5	93.0	23.2	88	2.2	8.7	0.81	0.12	0.010
CS24	f	3.5	103.0	29.0	82	2.5	5.6	0.65	0.10	0.0083
CS25	f	4.5	104.5	38.7	88	3.4	11	0.66	0.16	0.0076
CS26	f	4.5	102.0	30.0	87	4.9	7.7	0.67	0.12	0.0057
CS27	f	7.5	111.0	39.0	91	5.6	11	0.57	0.14	0.015
CS28	f	7.5	126.0	55.5	82	4.7	18	0.51	0.22	0.011
CS30	p	9.5	104.5	55.9	73	2.8	7.5	0.54	0.09	0.0091
CS31	p	12.5	112.0	58.0	90	4.5	11	0.71	0.063	0.0093
CS32	f	14.5	113.0	46.5	88	7.4	7.1	0.13	0.093	0.0086
CS33	f	16.5	106.5	31.8	90	8.7	23	1.0	0.33	0.010
CS34	p	17.5	123.0	59.5	74	5.1	10	0.36	0.085	0.011
CS35	f	20.5	114.0	34.3	91	15	43	1.5	0.49	0.010
CS36	f	23.5	111.5	41.2	91	7.3	25	0.98	0.24	0.011
CS37	p	25.5	112.5	45.5	75	2.2	7.2	0.25	0.084	0.015
CS38	f	26.5	111.0	34.3	93	13	38	1.4	0.40	0.013
CS39	f	26.5	110.5	26.5	91	23	88	2.0	0.50	0.0036
CS40	p	28.5	118.0	58.5	85	10	18	0.58	0.26	0.0045
CS41	f	29.5	117.5	53.8	90	4.5	16	0.42	0.20	0.077
CS42	f	31.5	120.0	47.0	97	4.3	19	0.59	0.10	0.0046
CS43	f	41.5	117.5	45.2	81	2.8	6.9	0.30	0.10	0.0024
CS44	p	un	122.0	55.0	93	5.3	14	0.70	0.13	0.0081
Diet Fish (Roach: *Rutilus* sp.)										
FI01	un	un	16.7	0.096	7.9	0.033***	0.024	0.010	0.0016	0.0016
FI02	un	un	11.7	0.032	2.6		0.011	0.0027	0.0008	0.0003
FI03	un	un	12.8	0.041	4.8		0.017	0.0037	0.0008	0.0005

* m: male; f: female; p: pregnant female, un: unknown
** BL: body length, BW: body weight
*** Three animals were pooled and analyzed

Arctic and Antarctic, indicating that PCB pollution in the Caspian Sea is not so considerable, compared with those in developed nations such as Europe and North America.

The mean concentrations of CHLs and HCB were 0.25 μg/g and 0.011 μg/g, respectively, in mature male Caspian seals. The mean CHLs levels found in this species was comparable to those in ringed seal (*Phoca hispida*) from the Canadian Arctic (mean concentration in males were reported at 0.46 μg/g wet weight), which is a relatively pristine area (Muir *et al.* 1988), implying relatively smaller usage of this pesticide around the Caspian Sea. The mean concentration of HCB in adult male Caspian seals was 11 ng/g on wet-weight basis. This level was similar to those in ringed seals from Norwegian or Russian Arctic (Luckas *et al.* 1990; Nakata *et al.* 1998). Remote cold

Table 2. Comparison of organochlorine concentrations (mean and range in parentheses, µg/g wet weight) in the blubber of male Caspian seals with those in other seals collected in 1980s and 1990s from various areas

Species	Location	Year	n	Age (years)	Fat (%)	PCBs	DDTs	HCHs	Reference
Caspian seal	Caspian Sea	1993	10	0.5–4.5	84 (80–90)	5.7 (3.2–9.5)	14 (7.2–22)	1.0 (0.56–1.3)	This study
Caspian seal	Caspian Sea	1993	6	6.5–23	85 (71–91)	9.7 (4.2–15)	19 (8.6–30)	1.3 (0.86–1.9)	This study
Baikal seal	Lake Baikal	1992	16	8.5–36	87 (81–94)	27 (9.4–56)	55 (14–140)	0.077 (0.033–0.13)	Nakata *et al.* 1995
Ringed seal	Barrow Strait, Canadian Arctic	1984	19	10	90 (NA)	0.57 (NA)	0.71 (NA)	0.27 (NA)	Muir *et al.* 1988
Ringed seal	Kara Sea, Russian Arctic	1995	29	1.9–9.5	89 (73–93)	4.2 (1.9–11)	3.6 (1.1–14)	0.18 (0.066–0.37)	Nakata *et al.* 1998
Grey seal	Baltic Sea	1982–88	5	9–20	90 (87–92)	110* (66–180)*	33* (11–54)*	NA	Roos *et al.* 1992; Blomkvist *et al.* 1992
Ringed seal	Baltic Sea	1981–86	5	20–40	89 (87–92)	320* (150–770)*	340 (160–820)*	NA	Roos *et al.* 1992; Blomkvist *et al.* 1992
Harbor seal	Skagerrak	1988	4	5.0–7.0	86 (80–89)	47* (28–82)*	8.4* (7.0–10)*	NA	Roos *et al.* 1992; Blomkvist *et al.* 1992
Harbor seal	Skagerrak	1988	4	10–22	84 (81–87)	84* (47–110)*	7.0* (4.3–11)*	NA	Roos *et al.* 1992; Blomkvist *et al.* 1992
Harbor seal	Wadden Sea	1988	3	5	70 (64–79)	29 (24–35)	2.1** (1.3–2.5)	NA	Storr-Hansen and Spliid 1993b
Monk seal	Mediterranean Sea	1990	1	6+	87	15	17	0.027***	Menchero *et al.* 1994
Larga seal	Western coast of North Japan	1994	1	Adult	74	12	19	0.73	Watanabe *et al.* 1999
Larga seal	Western coast of North Japan	1992–94	3	Immature	76 (71–80)	7.4 (3.2–12)	7.9 (3.4–13)	1.1 (0.58–1.6)	Watanabe *et al.* 1999
Larga seal	Sea of Okhotsk, Japan	1996	8	Adult	83 (67–89)	3.2 (1.4–6.5)	2.8 (1.5–5.0)	0.33 (0.21–0.66)	Nakata *et al.* 1998
Weddel seal	Showa Station, Antarctic	1981	1	13 or 14	88	0.043	0.19	NA	Hidaka *et al.* 1984
Ross seal	Queen Maud Land, Antarctic	1981/82	3	NA	NA	0.039 (0.005–0.095)	0.088 (0.047–0.12)	NA	McClurg 1984

NA: No data available
* Lipid weight basis
** *p,p′*-DDE only
*** α- and γ-isomers only

waters have been reported to be contaminated by HCB, due to the dispersible nature of HCB through long-range atmospheric transport (Calamari *et al.* 1991). HCB concentrations in cetaceans from cold waters were noted to be comparable or slightly higher than those from temperate and tropical regions (Prudente *et al.* 1997; Aono *et al.* 1998). The contamination pattern of HCB found in Caspian seals is likewise reflective of this global distribution trend.

Age- and Sex-Dependent Accumulation

Figure 1 shows the variations in organochlorine concentrations with age in Caspian seals. Except HCB, organochlorine concentrations in male animals slightly increased with age. The decreasing trend of HCB residues might have been due to its biodegradability and less persistent nature of this chemical in Caspian seals.

Interestingly, differences in organochlorine concentrations between adult males and females of Caspian seal were less (Figure 1). Several studies have reported that female animals retained lower concentrations of organochlorines than males in various aquatic mammals, such as Dall's porpoise (*Phocoenoides dalli*), short-finned pilot whale (*Globicephala macrorhynchus*), Baikal seal (*Phoca sibirica*), and ringed seal, due to the transfer of organochlorine residues from mothers to their pups during lactation (*e.g.,* Subramanian *et al.* 1987; Tanabe *et al.* 1987a; Nakata *et al.* 1995, 1998). The lactation period of Caspian seals (4–5 weeks), which is a dominant factor determining the male-female difference of organochlorine residue levels (Subramanian *et al.* 1987), was comparable to some other species such as ringed seal (5–7 weeks) and larga seal (*Phoca largha:* 4 weeks) (Bonner 1984).

An alternative explanation for the comparable concentrations of oragnochlorine residues in male and females may be the higher pregnancy failure rate in Caspian seal. Khuraskin and Pochtoyeva (1997) reported that the annual pregnancy failure rate in adult female Caspian seals was from 47 to 71%. In the present study, 65% of mature females collected were not pregnant (Table 1), which was higher than in Baikal seal (12%), beard seal (*Erignathus barbatus:* > 25%),

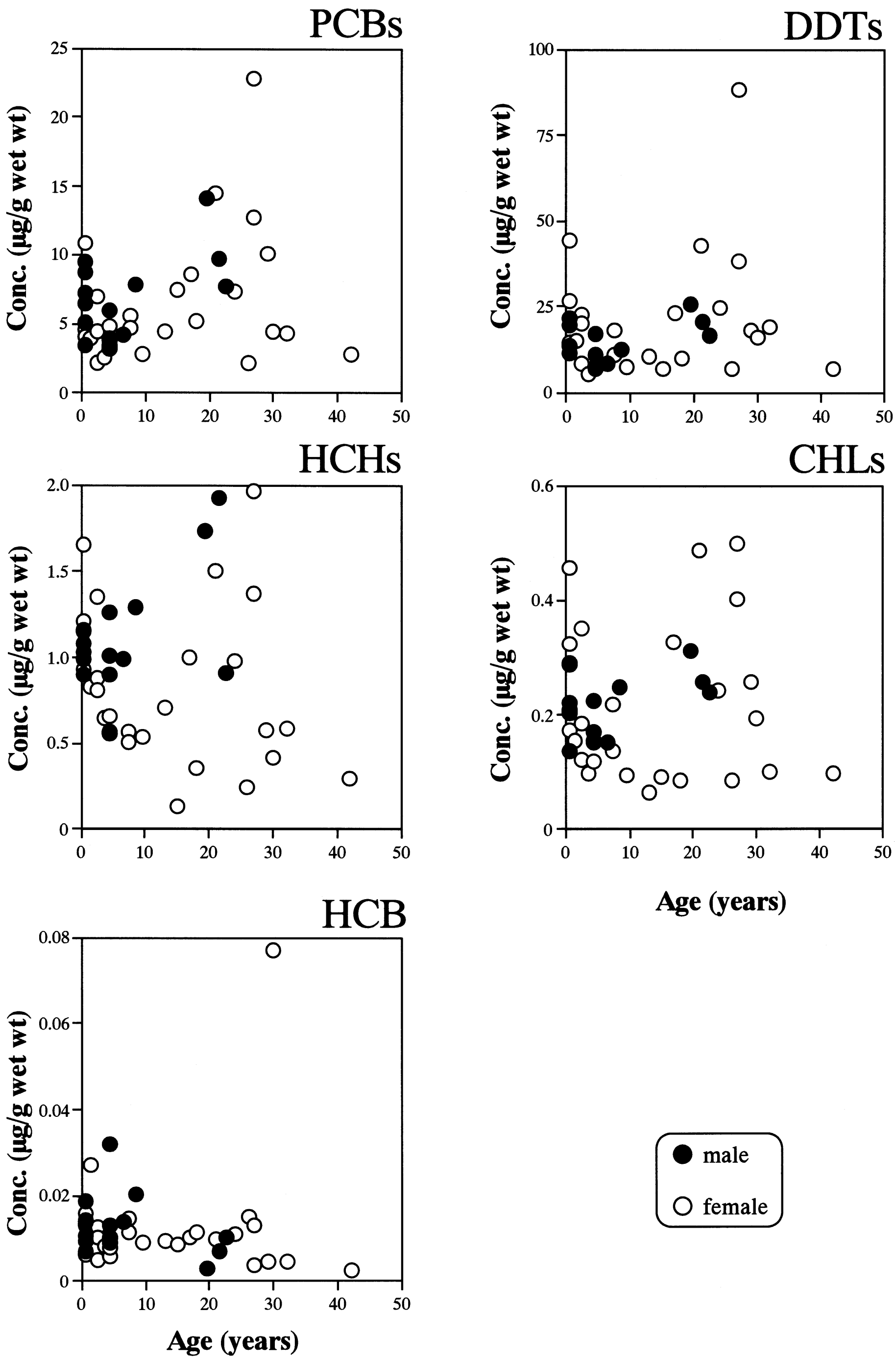

Fig. 1. Variation of the organochlorine concentrations with age in Caspian seals

harp seal (*Phoca groenlandica:* > 20%), and ringed seal (< 30%) from Russian waters (Popov 1982). Hence, these observations imply that lesser gender difference in organochlorine concentrations found in Caspian seals reflects lesser lactational and gestational excretion of organochlorines from the body of adult females due to the high rates of pregnancy failure.

The ranges of organochlorine concentrations in immature Caspian seals were wide with the higher levels similar to those of adult males (Figure 1), which decreased with body length (Figure 2). Furthermore, compositions of tetrachlorobiphenyls (TeCBs) and α-HCH to total PCBs and HCHs increased with growth in immature animals (Figure 2). These results imply that organochlorine concentrations and their compositions in the blubber of immature Caspian seals were affected by high concentrations of organochlorines in their mothers and by their growth due to high feeding rate. As previously mentioned, some adult female Caspian seals retained higher concentrations of organochlorine residues similar to or higher than adult males (Figure 1), which is probably due to the high rate of unsuccessful pregnancy. Such high levels of organochlorines in the body of adult females would consequently elevate organochlorine

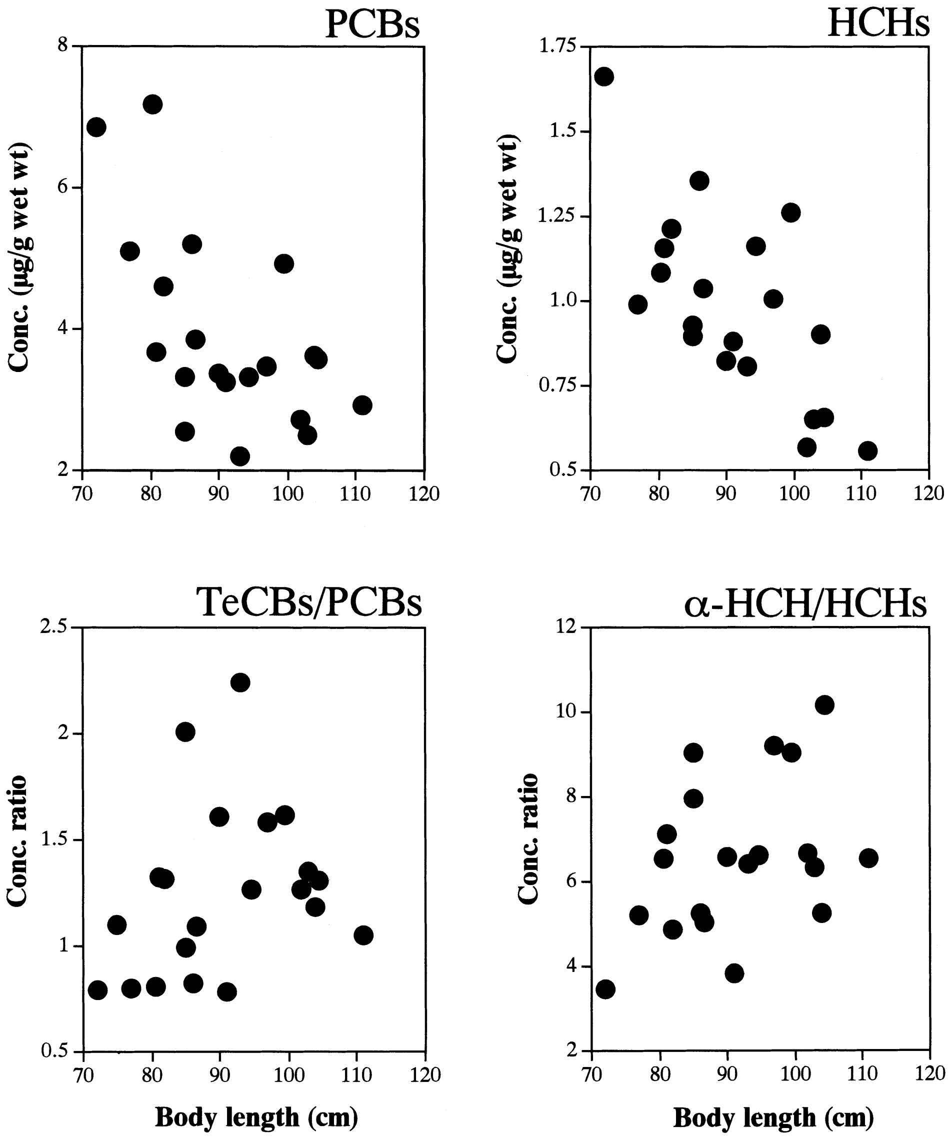

Fig. 2. Trend of concentrations of PCBs and HCHs and the concentration ratioes of tetrachlorobiphenyls (TeCBs)/PCBs and α-HCH/HCHs with body length of immature Caspian seals

concentrations in the body of their pups through gestation and lactation, when adult females successfully reproduce. Thereby, high residue levels of organochlorines are likely to be reflected in the weaning pups. Due to the high feeding rate in postnatal animals, the rapid growth could increase the dilution of organochlorine residue levels in the body of immature Caspian seals, resulting in a change in the composition of isomers such as TeCBs and α-HCH, which were predominant in their fish diet (Figure 3).

Composition and Metabolic Capacity

Compositions of PCB congeners, HCH isomers, DDT compounds, and CHL compounds in immature and mature Caspian seals and their diet fish are shown in Figure 3. Except for DDTs, relatively persistent congeners or derivatives (such as hepta- and octa-CBs, β-HCH, and oxychlordane) dominated in Caspian seals, whereas diet fish retained relatively less persistent compounds (such as penta-CBs, α-HCH, *trans*- and *cis*-chlordane), which constitute a larger proportions in commercial preparations. Percentages of persistent congeners and compounds of PCBs, HCHs, and CHLs were smaller in immature seals than adults (Figure 3). This is due to the greater influence of compositions in diet fish to immature animals, because fish retained considerable amounts of less persistent congeners and compounds and the body burdens of organochlorines in immature Caspian seals are lower than those in mature ones. Interestingly, percentage of *p,p′*-DDE was comparable in immature and mature seals and their diet fish (Figure 3). Although the reason for this is yet unclear, this may be suggestive of the decline in recent input of DDT in Caspian Sea.

For understanding the degradation capacity of organochlorines in Caspian seals, Metabolic Indices (MI) of PCB isomers and congeners in Caspian seals were examined following the method of Tanabe *et al.* (1988). In this context, PCB isomers and congeners were classified into the three groups as follows

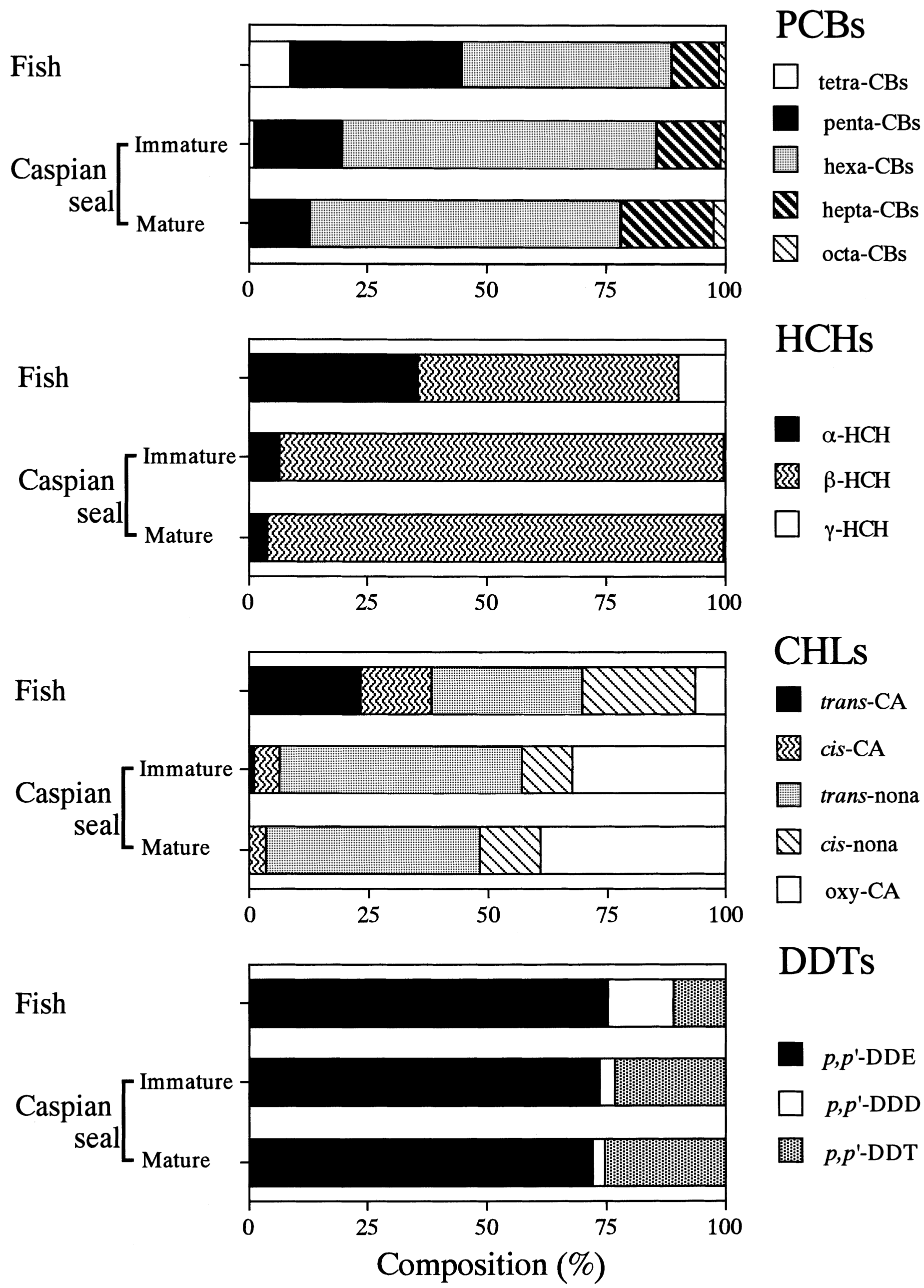

Fig. 3. Composition of PCB congeners, HCH isomers, CHL compounds, and DDT compounds in immature and mature Caspian seals and their diet fish

(Figure 4); Group I: no vicinal H-atoms in both *meta-para* and *ortho-meta;* Group II: unsubstituted Cl-atoms in vicinal position(s) of *meta-para;* and Group III: more than one area which has unsubstituted Cl-atoms in vicinal position(s) of *ortho-meta.* Group III was further classified into three subgroups according to the number of Cl-atoms in *ortho* position of biphenyls. Group III-1 have congeners with no Cl-atoms in *ortho* position. Group III-2 and Group III-3 have one and two Cl-atoms in *ortho* position, respectively. Earlier studies have pointed out that Group I is the persistent congener in the body of animals, and Group II and Group III are metabolized by CYP2B and CYP1A isozymes, respectively (Tanabe *et al.* 1988; Boon *et al.* 1994; Kannan *et al.* 1995). In Caspian seals, Group I congeners revealed much lower values of MI, indicating less degradability of congeners similar to those with other marine mammals (Tanabe *et al.* 1988; Boon *et al.* 1994; Kannan *et al.* 1995; Muir *et al.* 1996). MI values of Group II in Caspian seals were higher than those of Group I, which reflects the action of CYP2B isozymes in this seal. Compared with other aquatic mammals, MI values of Group II in Caspian seals (mean and range = 1.2 and 0.91–1.6) were higher than those of cetaceans (0.076 and −0.25–1.0: Tanabe *et al.* 1988), but similar to those of Baikal seals (1.0 and 0.20–1.9: Nakata *et al.* 1995). These results suggest that Caspian seals have higher capacity to degrade Group II congeners than cetaceans, but similar capacity to Baikal seal, a closely related species. MI values of Group III decreased with increasing number of Cl-atoms in *ortho*-positions of biphenyl rings. From these observations, it is

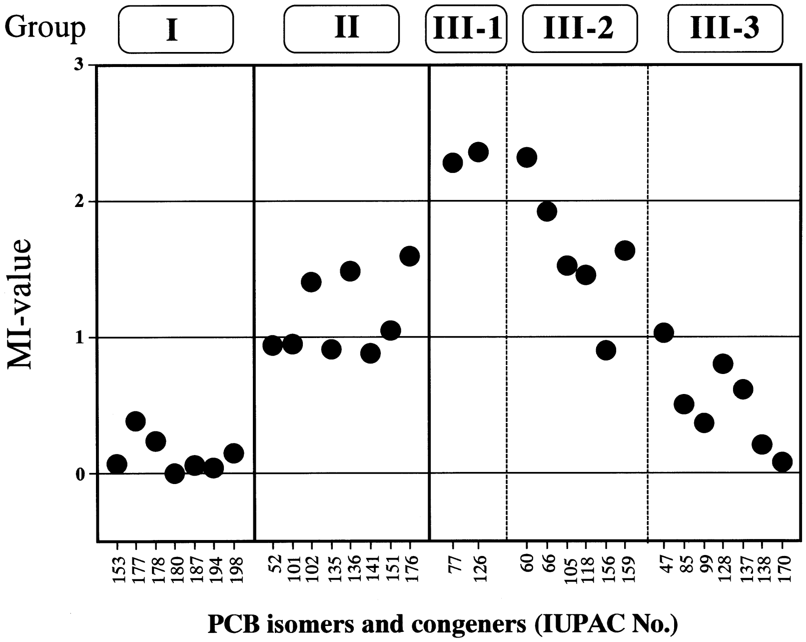

Fig. 4. Metabolic Indices (MI) of PCB congeners in adult male Caspian seals. Calculation of MI was obtained following the method of Tanabe *et al.* (1988)

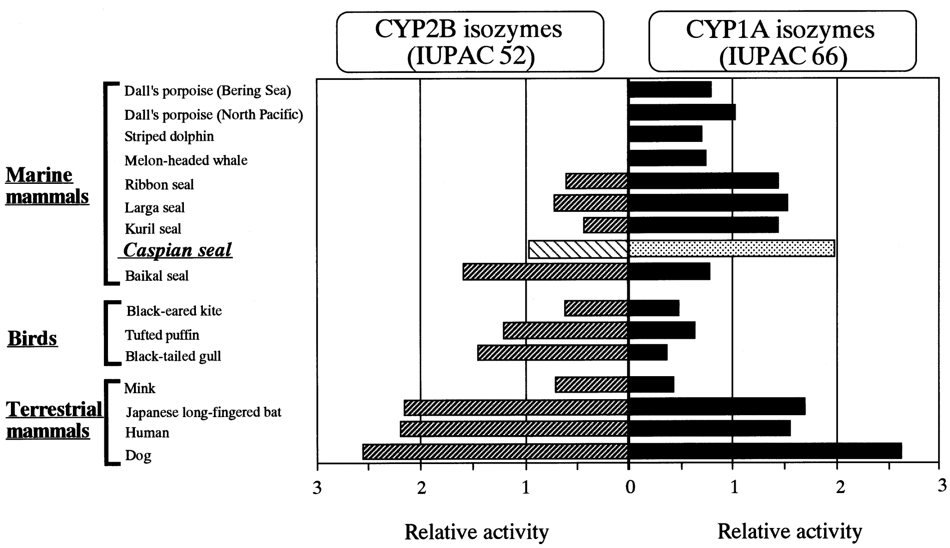

Fig. 5. Comparison of CYP2B and CYP1A isozyme activities in higher trophic animals as estimated by Metabolic Indices of IUPAC 52 and IUPAC 66, respectively. Data on Baikal seal and other animals were cited from Nakata *et al.* (1995) and Tanabe *et al.* (1988), respectively

noteworthy that Caspian seals have higher capacity to degrade non- and mono-*ortho* coplanar PCBs, which are known to exhibit the dioxin-like toxicity. Interestingly, as seen in Figure 4, this capacity was comparable or higher than the degradation of Group II congeners, which were metabolized by CYP2B isozymes. It has been pointed out that some cetaceans and seals could not degrade Group III-3 congeners (Boon *et al.* 1994; Kannan *et al.* 1995; Muir *et al.* 1996). However, Caspian seals showed significantly higher MI values of Group III-3 congeners than Group I ones (Figure 4), which means that Caspian seals have a capacity to degrade Group III-3 PCBs. Di-*ortho* coplanar PCBs (IUPAC 170 and 180) showed lower MI values as compared with non- and mono-*ortho* coplanar PCBs (Figure 4), indicating that di-*ortho* coplanar congeners have extremely less biodegradable nature in Caspian seals.

Following the methods employed by Tanabe *et al.* (1988), CYP2B and CYP1A isozyme activities in Caspian seals were estimated by MI values of IUPAC 52 and 66, respectively, and their values were compared with those in other higher animals (Figure 5). Apart from high CYP2B isozyme activity in Baikal seal, both CYP2B and CYP1A isozyme activities in Caspian seal were the highest among marine mammals. Surprisingly, CYP1A isozyme activity in Caspian seals was found to be comparable with those in terrestrial mammals. Muir *et al.* (1996) reported that beluga whale from St. Lawrence estuary have greater capacity to degrade PCB congeners than those from Arctic region, resulting from enzyme induction due to greater PCB exposure in St. Lawrence beluga whales. However, PCB residue levels in Caspian seals were not so high as compared with these of beluga whales (*Delphinapterus leucas*) from the St. Lawrence estuary and other seals

Table 3. Comparison of mean and range values of TEQs* (pg/g wet weight) in the blubber of Caspian seal with other seals from various waters

Species	Caspian Seal	Caspian Seal**	Baikal Seal	Harbor Seal	Ringed Seal	Ringed Seal	Harp Seal
n	43	1	40	40	7	4	10
Location	Caspian Sea	Caspian Sea	Lake Baikal***	Denmark***	Hudson Bay, Canada	Hudson Bay, Canada	Greenland Sea
Ref.	This study	This study	Nakata *et al.* (1997)	Storr-Hansen and Spliid (1993a)	Muir *et al.* (1995)	Muir *et al.* (1995)	Oehme *et al.* (1994)
non-*ortho* IUPAC							
77	0.41 ± 0.24 (0.11–1.2)	1.7	6.8 ± 4.7 (1.3–20)	0.012 ± 0.005 (0.0060–0.027)	0.026 ± 0.015 (0.012–0.053)	0.027 ± 0.015 (0.014–0.045)	0.085 ± 0.018 (0.065–0.11)
126	16 ± 6.6 (6.2–33)	66	310 ± 190 (61–900)	43 ± 10 (16–55)	18 ± 20 (4.4–57)	26 ± 27 (7.8–65)	14 ± 1.8 (13–17)
169	0.60 ± 0.24 (0.20–1.1)	1.7	2.9 ± 1.7 (0.20–7.8)	0.35 ± 0.25 (0.10–0.90)	0.18 ± 0.12 (<0.01–0.32)	0.34 ± 0.22 (0.082–0.57)	0.94 ± 0.86 (0.24–2.1)
mono-*ortho* IUPAC							
105	3.3 ± 1.4 (0.9–7.1)	15	49 ± 51 (6.5–270)	5.3 ± 2.3 (2.0–9.1)	3.3 ± 3.7 (0.54–11)	3.1 ± 1.4 (1.7–4.9)	0.82 ± 0.22 (0.58–1.1)
118	9.0 ± 3.7 (3.0–19)	52	120 ± 120 (6.1–580)	14 ± 6.0 (5.7–25)	6.5 ± 6.5 (1.3–21)	5.9 ± 2.7 (3.6–9.6)	2.6 ± 0.97 (1.6–3.6)
156	18 ± 8.5 (4.7–49)	130	75 ± 55 (13–310)	37 ± 22 (9.3–100)	4.3 ± 9.7 (0.75–14)	4.7 ± NA (1.5–7.5)	1.5 ± 0.7 (0.85–2.4)
di-*ortho* IUPAC							
170	2.8 ± 1.6 (0.63–7.5)	11	NA	44 ± 34 (8.6–140)	1.8 ± 1.7 (0.48–4.6)	2.0 ± 1.4 (0.60–3.8)	NA
180	1.2 ± 0.84 (0.3–3.5)	5.3	7.6 ± 8.0 (0.53–42)	9.3 ± 6.9 (1.1–29)	NA	NA	0.22 ± 0.073 (0.13–0.31)
ΣTEQs	51 ± 20 (18–140)	280	570 ± 350 (160–1800)	140 ± 76 (46–360)	34 ± 42 (7.5–110)	42 ± NA (15–91)	21 ± 2.9 (17–24)

NA: No data available
* Data indicate mean ± SD and (range)
** This seal (CS39) retained PCBs with extraordinarily high concentrations
*** Mass mortality of seals was recorded

from highly polluted areas (Table 2). Thus, higher degradation capacity found in Caspian seals is unlikely to be induced by PCB exposure. Engelhardt (1982, 1983) reported that drug-metabolizing enzymes, especially CYP1A, were induced in oil-exposed marine mammals including seals. Considering the extensive oil production and its increasing accidental leakage in Caspian Sea (Karphinsky 1992), the enzyme induction by petroleum chemicals such as PAHs might be related to the higher capacity to degrade PCBs in Caspian seals.

Toxic Assessment

Table 3 shows mean and range of TEQ values for non-, mono-, and di-*ortho* coplanar PCBs in Caspian seals and other seals from various waters. TEQs were estimated using TEF of Ahlborg *et al.* (1994). Mean and range of ΣTEQs in Caspian seals were 51 pg/g and 18–140 pg/g (wet weight), except for a seal of CS39. ΣTEQs in CS39 were extraordinarily high compared with those in other Caspian seals. Except for this animal, ΣTEQs in Caspian seals were apparently lower than those in Baikal seals from the Lake Baikal and immature harbor seals (*Phoca vitulina*) from the North Sea, where mass mortality has taken place in the past (Table 3). Furthermore, range and mean of ΣTEQs in Caspian seals were comparable to those noted in seals from the Canadian Arctic and in larga seal from the Sea of Okhotsk (mean TEQs were reported on 42 pg/g on lipid weight) (Nakata *et al.* 1998). These observations might be attributable to the lesser exposure to PCBs and high degradation capacity for coplanar PCBs in Caspian seals as discussed earlier. Lower TEQs in Caspian seals imply that the effect of coplanar PCBs is not a prominent factor for the abnormal mortality that took place in 1997 in Caspian Sea.

In Caspian seal, TEQs of non- and mono-*ortho* coplanar PCBs occupied 33% and 59% in ΣTEQs, whereas those of di-*ortho* coplanar PCBs were only 7.8% (Table 3). Among non- and mono-*ortho* coplanar PCBs, TEQs of IUPAC 126 and 156 were comparable with the highest contribution in this seal, followed by IUPAC 118, 105, 169 and 77 (Figure 6). Compared to TEQ contribution in other seals, the lowest percentage of non-*ortho* coplanar PCBs, but higher proportion of IUPAC 156 in Caspian seals was observed (Figure 6). This pattern was rather close by similar to those in cetaceans. Such a unique pattern of TEQ composition found in Caspian seals seems to have risen from high activity of CYP1A

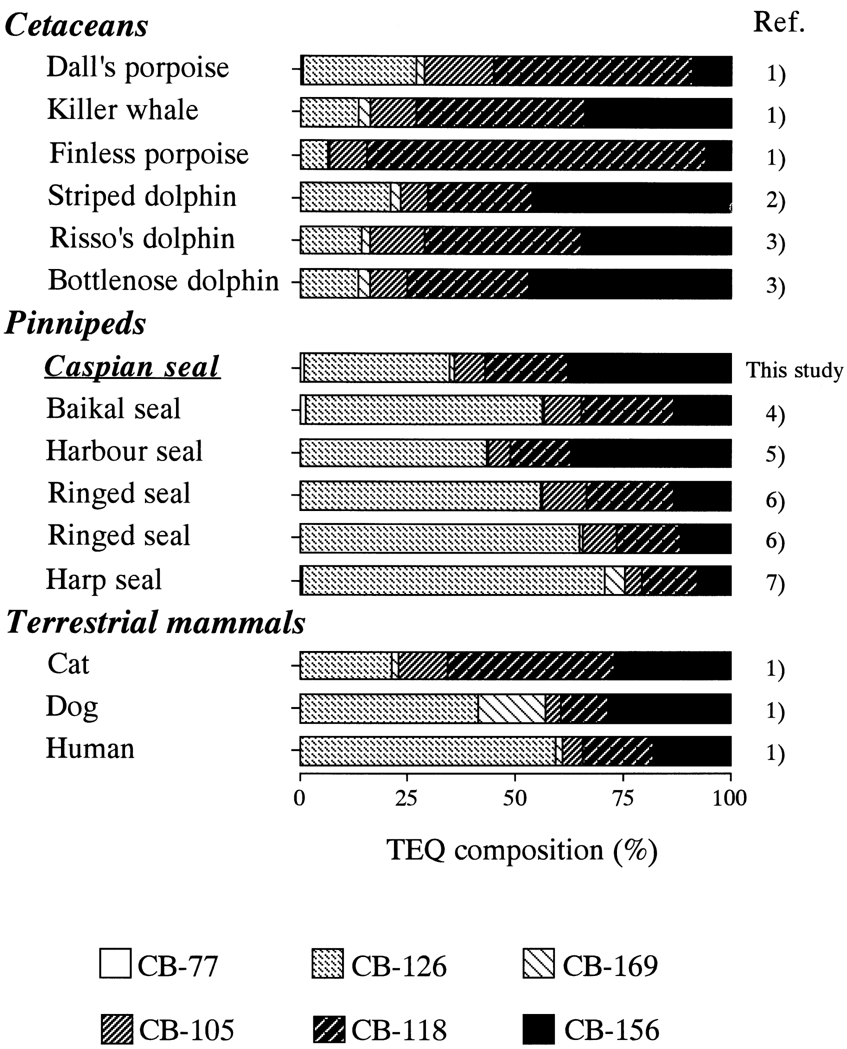

Fig. 6. Comparison of TEQ compositions of non- and mono-*ortho* coplanar PCBs in Caspian seal and those in other higher trophic animals. Data cited from (1) Kannan *et al.* (1989); (2) Kannan *et al.* (1993); (3) Corsolini *et al.* (1995); (4) Nakata *et al.* (1997); (5) Storr-Hansen and Spliid (1993a); (6) Muir *et al.* (1995); (7) Oehme *et al.* (1994)

isozymes, which have a potency to degrade non-*ortho* coplanar PCBs.

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