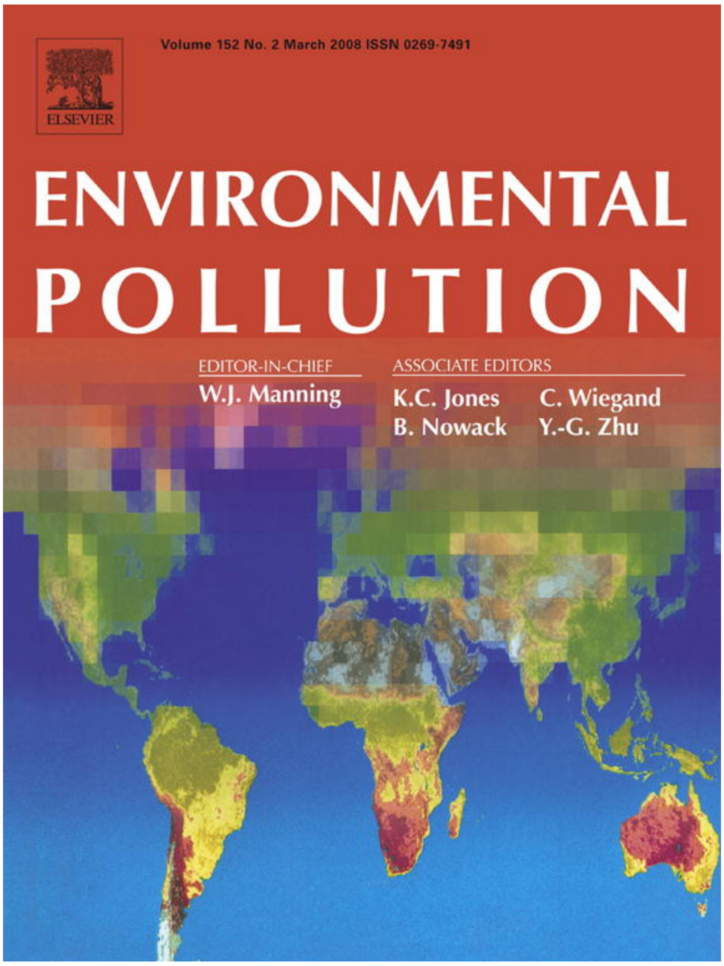
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Persistent organic pollutants (POPs) in Caspian seals of unusual mortality event during 2000 and 2001

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POPs in seals are high enough to induce immunosuppression.

Abstract

Persistent organic pollutants including organochlorine pesticides, PCBs, and PCDDs/DFs were determined in the blubber of Caspian seals, which died during an outbreak of canine distemper virus in 2000 and 2001. DDTs were the predominant contaminants that ranged from 3.1 to 560 μg/g lipid. A negative correlation was observed between concentration of contaminants and blubber thickness. During spring, as the blubber layer becomes thin after breeding and moulting, seals may face higher risk due to the increased concentration of organochlorines in their bodies. TEQs in the blubber of Caspian seals (10–340 pg TEQ/g) were lower than those in seals from other locations, suggesting that toxic effects of these contaminants are a deal less in the present population and they are unlikely to be linked to mass mortality. The levels of PCBs and pesticides in Caspian seals, however, comparable to those in other aquatic mammals that have suffered from epizootics, might pose a risk of immunosuppression.

Keywords: Persistent organic pollutants; Dioxins; Caspian seal; Mass mortality; Bony fish

1. Introduction

Since 1968, several abnormalities such as reproductive failure, immune impairment, tumors and virus infection have been observed in aquatic mammals over the world, and declining population and mass mortality have also been reported (reviewed by Colborn and Smolen, 1996). The latest event is a phocine distemper virus (PDV) outbreak in Northern Europe in 2002 that killed thousands of harbor seals (*Phoca vitulina*) (Jensen et al., 2002). Most of the unusual events have taken place near the areas with high industrial and human activities. Considerable concentrations of anthropogenic chemicals including persistent organic pollutants (POPs) were detected in aquatic mammals from these areas. In the Caspian Sea, unusually high mortality of Caspian seals (*Pusa caspica*), an apex predator in the ecosystem, started during the spring of 2000,

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and infection by canine distemper virus (CDV) was found to be the primary cause of mortality (Kennedy et al., 2000; Stone, 2000; Kuiken et al., 2006). Since organochlorine-induced higher susceptibility to viral diseases has been well documented in mammals (Koller and Thigpen, 1973; Imanishi et al., 1980; De Swart et al., 1996), high levels of organochlorine (OC) compounds in Caspian seals were suspected to be the major contributor to the mass mortality and thus demanding immediate research. In our previous study, OCs and organotin compound residues in limited number of seal samples were examined and found that even though contamination status of OCs in the Caspian Sea seemed to be improving, concentrations in seals at poor health condition were extremely high (Kajiwara et al., 2002).

In addition to our previously published data, many additional Caspian seal samples found dead during the epizootic in 2000 and 2001 were examined in this study to elucidate geographical and seasonal differences of contamination and the subsequent ecotoxicological risk by OCs including polychlorinated dibenzo-*p*-dioxins (PCDDs), furans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs). Bony fish samples, which are the prey species of seal, were also collected from Caspian Sea and analyzed in this study. This may also be useful in evaluating the regional contamination by OCs.

2. Materials and methods

2.1. Sample collection

Thirty-six male and 17 female Caspian seals affected by the outbreak of the CDV epizootic were used in this study. They were found stranded on the coasts of Iran, Azerbaijan, Dagestan Republic (Russia), Kazakhstan, and Turkmenistan during May 2000–June 2001. Biological data of the animals analyzed in the present study are given in Table 1. Blubber samples were excised from dead animals, wrapped in aluminium foil, and kept in a deep-freezer at −20 °C until analysis. For comparison, six Caspian seals collected from the Pearl Island, northwestern part of Caspian Sea during November 1993 were also used for analysis of dioxins and related compounds. All these animals were captured with permission of local government in Russia and immediately dissected on-board.

Bony fish samples were collected off the Caspian coasts of Kazakhstan, Azerbaijan, Turkmenistan, and Iran during 2000 and 2002 (Table 2). Six species of fish, Caspian roach (*Rutilus rutilus caspicus*), monkey goby (*Neogobius fluviatilis*), round goby (*Neogobius melanostomus*), Caspian sprat (*Clupeonella delicatula*), anchovy sprat (*Clupeonella engrauliformes*), and Caspian sand-smelt (*Atherina boyeri caspia*) were caught and stored in deep-freezer at −20 °C. Whole body samples of several specimens of the same species were pooled and homogenized using a homogenizer for chemical analysis.

2.2. Chemical analysis

PCBs, DDT (dichlorodiphenyltrichloroethane) and its metabolites, HCHs (hexachlorocyclohexane isomers), CHLs (chlordane related compounds), HCB (hexachlorobenzene), heptachlor epoxide (HP-epox.), TCPMe [tris (4-chlorophenyl) methane] and TCPMOH [tris (4-chlorophenyl) methanol] were analyzed following the method described previously (Kajiwara et al., 2003) with slight modifications. Briefly, approximately 2–3 g of blubber or 20 g of fish sample was ground with anhydrous sodium sulfate and extracted in a Soxhlet apparatus with a mixture of diethyl ether and hexane. After concentration of the extract, an aliquot of extract was added to a gel permeation chromatography (GPC: Bio-Beads S-X3, Bio-Rad Laboratories, CA, USA, 2 cm id. and 50 cm length) column for lipid removal. The GPC fraction containing OCs was concentrated and passed through an activated Florisil (Florisil PR: Wako Chemicals USA, Inc., USA) column for clean-up and fractionation. Quantification of PCBs and most of OC pesticides was performed using a gas chromatograph (GC: Agilent 6980 N) equipped with a micro-electron capture detector (micro-ECD) and an auto-injection system (Agilent 7683 Series Injector). The GC column used for OC analysis was a fused silica capillary (DB-1, 30 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific Inc.). Identification and quantification of HP-epox., TCPMe and TCPMOH were performed using a GC with a mass selective detector (MSD: Agilent 5973 N) in selective ion monitoring (SIM) mode equipped with an auto-injection system (Agilent 7683 Series Injector). OC concentrations were calculated from the peak area of the sample to the corresponding external standard. The PCB standard used for quantification was an equivalent mixture of Kanechlor preparations (KC-300, KC-400, KC-500 and KC-600) with known PCB composition and content. Concentrations of individually resolved peaks of PCB isomers and congeners were summed to obtain total PCB concentrations.

Analysis of PCDDs/DFs and DL-PCBs was performed following the procedure described by Tanabe et al. (2004). Briefly, blubber samples (5–6 g) were extracted in a Soxhlet apparatus with dichloromethane. $^{13}C_{12}$-labeled PCDDs/DFs and DL-PCBs (2,3,7,8-T_4CDD/DF, 1,2,3,7,8-P_5CDD/DF, 1,2,3,6,7,8-H_6CDD/DF, 1,2,3,7,8,9-H_6CDD/DF, 1,2,3,4,6,7,8-H_7CDD/DF, O_8CDD/DF, CB77, CB81, CB118, CB126, CB156, CB167, CB169, and CB189) were spiked into the concentrated extract as internal standards. Lipid in this solution was removed by GPC. The GPC fraction containing PCDDs/DFs and DL-PCBs was concentrated and then passed through activated silica-gel (Wakogel S-1: Wako Pure Chemical Industries, Ltd., Japan) packed in a glass column for clean-up. Separation of PCDDs/DFs and DL-PCBs fraction was performed by passing through activated alumina (Aluminium oxide 90 active basic: Merck KGaA, Germany) and activated carbon-dispersed silicagel (Kanto Chemical Co., Inc., Japan) packed in a glass column. As a recovery spike, $^{13}C_{12}$-labeled CB105, CB157, and CB180 were added to the mono-*ortho* DL-PCB fraction, and $^{13}C_{12}$-labeled 1,2,3,4-T_4CDD and 1,2,3,7,8,9-H_6CDD were added to the fraction for non-*ortho* DL-PCBs and PCDDs/DFs. Identification and quantification of PCDDs/DFs and non-*ortho* DL-PCBs were performed using a GC (Agilent 6890 Series) with a high resolution MSD (JEOL JMS-700D, JEOL, Japan), and mono-*ortho* DL-PCBs using a GC with a bench-topped double-focusing MSD (JEOL GCmate II, JEOL, Japan). All the congeners were quantified using isotope dilution method to the corresponding $^{13}C_{12}$-congeners. Recovery of $^{13}C_{12}$-labeled PCDDs/DFs and DL-PCBs ranged between 60 and 120%.

Procedural blanks were analyzed simultaneously with every batch of five samples to check for interferences or contamination from solvents and glassware. Lipid contents were determined by measuring the total non-volatile solvent extractable material on sub-samples taken from the original extracts. The concentrations of OCs are expressed on a lipid weight basis unless otherwise specified.

For quality assurance and control, our laboratory participated in the Intercomparison Exercise for Persistent Organochlorine Contaminants in Marine Mammals Blubber, organized by the National Institute of Standards and Technology (Gaithersburg, MD) and the Marine Mammal Health and Stranding Response Program of the National Oceanic and Atmospheric Administration's National Marine Fisheries Service (Silver Spring, MD). Standard reference material (SRM 1945) was analyzed for selected PCB congeners and persistent OCs. Data from our laboratory were in good agreement with those for reference materials. The average of percentage deviation from the certified values was 13% (range: 0.5–20%) for OC pesticides and 28% (range: 1.3–57%) for PCB congeners.

Statistical analyses were executed using program STATCEL (OMS Publishing Inc.). Probability values less than 0.05 were considered as significant using Mann–Whitney *U* test and Spearman's rank correlation.

3. Results and discussion

3.1. Contamination status of PCBs and OC pesticides

OC contaminants were detected in all the blubber samples of Caspian seals stranded during 2000 and 2001 (Table 1).

Table 1
Biological data and OC concentrations in the blubber of Caspian seals collected in 2000 and 2001

Sample ID	Sex[a]	BL[b] (cm)	BT[c] (cm)	Lipid (%)	PCBs	DDTs	HCHs	CHLs	HP-epox.[d]	HCB	TCPMOH	TCPMe
					(μg/g lipid weight)				(ng/g lipid weight)			
Iran												
CS-Iran-A	M	117	5.5	88	2.6	9.5	0.40	0.12	34	6.4	35	4.7
CS-Iran-B	M	128	2.5	34	36	130	6.3	1.8	560	29	900	50
CS-Iran-G	M	130	4.5	91	7.0	30	0.76	0.30	95	4.7	110	16
CS-Iran-H	M	133	5.0	89	11	41	1.1	0.59	150	4.9	250	20
CS-Iran-E	M	134	2.0	74	28	140	2.0	0.89	240	12	770	140
CS-Iran-R	M	139	4.5	81	18	55	0.63	0.76	94	6.8	120	18
CS-Iran-S	M	140	4.0	80	6.6	30	0.71	0.40	94	2.9	89	13
CS-Iran-J	M	142	5.5	91	11	68	0.72	0.53	110	3.7	170	38
CS-Iran-T	M	142	2.0	63	13	33	0.88	0.32	77	7.2	190	42
CS-Iran-L	M	144	5.5	90	2.1	5.2	0.11	0.057	11	1.2	12	25
CS-Iran-M	M	144	5.5	70	17	55	0.79	0.72	130	8.9	280	43
CS-Iran-Q	M	144	3.5	83	9.5	34	0.57	0.36	62	5.2	39	17
CS-Iran-K	M	146	7.0	89	2.8	6.7	0.23	0.070	20	6.6	15	15
CS-Iran-D	M	150	6.0	90	5.5	25	0.54	0.20	49	2.9	61	26
CS-Iran-O	M	151	6.5	90	15	43	0.86	0.48	85	7.8	420	51
CS-Iran-P	F	123	1.5	72	11	31	2.7	0.66	230	36	120	11
CS-Iran-F	F	134	4.0	78	1.9	4.5	0.17	0.057	16	1.2	7.3	2.5
CS-Iran-N	F	136	5.0	83	3.2	5.8	0.26	0.11	21	7.0	14	5.4
CS-Iran-I	F	138	6.5	85	1.5	4.9	0.13	0.061	12	3.2	7.5	3.3
CS-Iran-C	F	145	4.0	87	8.9	30	0.94	0.38	100	7.7	170	13
Azerbaijan												
Baku 6	M	77	0.7	72	26	64	3.0	1.3	310	160	710	22
CS-AZ-00-#22	M	90	2.0	68	2.7	7.3	0.47	0.13	40	19	24	1.8
CS-AZ-01-#16	M	90	2.0	89	2.1	3.1	0.18	0.068	19	9.4	16	1.5
Baku 25	M	105	0.8	82	27	67	5.3	1.9	640	32	530	19
Baku 32	M	110	0.9	84	35	120	3.8	2.6	620	32	750	45
Baku 1	M	112	0.7	37	320	470	9.9	14	2600	10	9900	950
CS-AZ-01-#13	M	121	1.5	51	30	89	3.4	1.4	320	22	450	18
CS-AZ-01-#15	M	121	0.8	77	46	200	3.6	2.0	390	8.4	720	76
CS-AZ-01-#11	M	122	1.5	67	36	110	2.9	1.9	410	11	410	45
CS-AZ-00-#07	M	123	2.0	62	110	520	13	5.2	1500	24	2100	210
Baku E	M	125	1.0	84	24	130	5.3	2.3	1000	27	700	57
CS-AZ-01-#14	M	130	2.0	64	24	74	2.5	1.8	410	17	520	56
CS-AZ-01-#06	M	132	1.5	88	23	120	2.3	0.93	210	120	210	39
CS-AZ-01-#08	M	132	2.0	91	21	110	2.1	1.3	340	6.7	110	47
CS-AZ-01-#12	M	132	1.0	80	39	170	2.7	2.5	440	29	1300	55
CS-AZ-01-#04	M	137	1.0	83	50	210	3.4	3.6	610	16	540	180
Baku 5	F	80	2.1	93	2.4	6.3	0.82	0.21	63	17	28	1.0
Baku 4	F	83	1.9	96	4.1	13	0.69	0.25	63	24	72	3.3
Baku 2	F	106	1.5	82	10	17	0.72	0.30	67	3.8	63	46
Baku 3	F	107	0.0	25	120	230	5.3	4.8	610	24	820	310
CS-AZ-01-#07	F	117	2.5	91	4.0	10	0.62	0.18	54	6.9	18	2.4
CS-AZ-01-#09	F	122	1.5	82	13	34	1.9	0.58	150	16	50	7.8
CS-AZ-01-#05	F	123	1.2	84	24	56	2.8	1.0	350	15	320	51
CS-AZ-01-#10	F	124	2.0	86	4.8	10	0.34	0.15	30	5.0	18	4.9
Dagestan Republic												
CS0002	M	nm[e]	nm	27	55	150	5.3	1.6	420	7.8	170	120
CS0006	M	nm	nm	23	150	430	17	5.9	1500	19	2600	190
CS0014	M	nm	nm	36	57	140	4.9	1.6	390	10	600	110
CS0011	F	nm	nm	27	120	560	11	7.2	1500	31	1100	300
Kazakhstan												
KZPC-2000-09	F	78	1.5	86	5.8	12	1.2	0.25	100	49	94	1.7
KZPC-2000-08	F	87	1.4	84	8.9	30	2.1	0.78	260	12	140	6.2
KZPC-2000-10	F	115	1.6	90	18	23	0.85	0.51	110	7.7	93	31
Turkmenistan												
CS-T1	M	89	1.2	81	3.5	9.5	0.89	0.21	68	14	60	1.4
CS-T2	M	117	1.5	73	32	160	3.0	2.3	480	14	300	43

[a] M, male; F, female.
[b] Body length, length from tip of nose to end of tail.
[c] Blubber thickness.
[d] Heptachlor epoxide.
[e] Not measured.

Table 2
Biological data and OC concentrations (ng/g lipid weight) in fish collected from the Caspian Sea in 2000 and 2001

Sample ID		*n*[a]	BL[b] (cm)	BW[c] (g)	Lipid (%)	PCBs	DDTs	HCHs	CHLs	HCB
Kazakhstan September 2001										
N 20	Caspian sand-smelt	15	9.6 (8.1–11)	4.6 (2.5–7.2)	4.2	220	170	16	6.4	2.1
N 31	Caspian sand-smelt	15	11 (9.9–12)	6.6 (5.2–8.8)	5.1	160	86	13	3.7	2.0
N 12	Caspian sprat	35	5.6 (4.9–6.5)	0.75 (0.49–1.2)	3.5	130	42	6.8	3.2	3.9
N 21	Caspian sprat	100	5.5 (4.6–7.3)	0.71 (0.36–1.5)	2.9	190	69	12	4.9	4.9
N 37	Caspian sprat	42	6.9 (5.0–9.6)	1.6 (0.58–5.3)	4.3	160	89	11	4.7	3.7
N 29	Caspian sprat	240	5.9 (4.7–8.1)	0.89 (0.37–2.7)	5.5	140	65	11	3.9	4.4
N 28	Monkey goby	32	8.4 (7.0–9.9)	4.9 (2.8–7.3)	1.8	150	64	16	5.4	4.5
N 7	Monkey goby	6	11 (10–12)	12 (8.4–19)	1.2	220	110	22	8.4	5.3
N 39	Monkey goby	20	9.7 (8.6–12)	8.2 (4.5–14)	1.5	170	72	15	5.5	5.0
N 24	Caspian roach	3	17 (13–20)	62 (40–96)	2.8	310	200	18	7.9	6.9
N 15	Caspian roach	4	11 (8.0–15)	29 (7.6–67)	1.4	260	160	14	7.6	6.2
N 18	Caspian roach	9	12 (10–19)	16 (7.6–68)	2.4	210	130	15	6.9	5.8
N 9	Caspian roach	4	11 (9.2–15)	26 (11–61)	3.7	150	76	13	5.4	3.3
N 2	Caspian roach	3	15 (15–16)	60 (54–69)	2.6	260	140	19	5.2	5.4
Azerbaijan October 2000										
s2-1	Caspian sprat	10	8.8 (7.8–9.7)	2.9 (2.0–3.8)	6.1	160	160	25	9.3	2.4
s2-2	Caspian sprat	10	8.6 (7.8–9.9)	3.1 (2.4–3.8)	6.9	210	210	32	8.7	6.3
s2-3	Caspian sprat	10	9.2 (8.5–9.9)	3.8 (2.8–5.2)	7.2	290	260	27	9.9	7.7
g1	Round goby	3	11 (10–13)	nm[d]	0.62	500	220	60	8.5	4.2
g2	Round goby	2	8.2 (7.5–8.8)	5.1 (4.0–6.2)	1.3	390	210	67	6.1	4.8
g4-1	Round goby	4	10 (9.9–11)	14 (12–15)	3.1	150	250	31	13	3.0
g4-2	Round goby	2	14 (12–16)	23 (17–29)	2.1	600	1100	53	36	9.0
g4-3	Round goby	3	11 (8.9–12)	15 (13–17)	2.4	320	420	51	17	6.3
g4-4	Round goby	2	11 (9.1–13)	20 (8.4–32)	3.1	300	390	30	16	5.3
Turkmenistan January–February 2002										
	Gobies	5	18 (16.5–18.5)	120 (94–140)	1.5	1800	990	99	9.9	12
	Caspian roach	6	17 (15.5–17.0)	84 (78–100)	11	140	180	45	4.3	5.0
	Anchovy sprat	29	8.9 (8.1–9.8)	4.6 (3.6–5.2)	3.5	200	330	56	9.2	8.7
Iran December 2001										
St. 1	Caspian roach	10	17 (16–20)	130 (92–170)	13	40	190	28	2.2	2.0
St. 2	Caspian roach	10	18 (17–19)	120 (100–140)	8.5	72	300	33	2.8	3.3
St. 3	Caspian roach	6	18 (16–20)	120 (92–160)	15	120	390	26	4.0	3.1
St. 4	Caspian roach	12	17 (16–19)	110 (94–160)	16	70	350	25	3.1	2.3
St. 5	Caspian roach	8	17 (16–18)	100 (91–110)	16	90	450	24	4.4	1.4
St. 1	Gobies	12	12 (10–14)	38 (21–63)	3.1	300	550	29	2.4	8.6
St. 2	Gobies	10	14 (13–15)	58 (47–72)	4.1	390	670	44	6.8	4.9
St. 3	Gobies	11	15 (9.8–22)	79 (16–200)	3.8	370	1100	63	13	8.0
St. 4	Gobies	2	21 (20–22)	170 (130–210)	3.5	900	1500	170	29	14
St. 5	Gobies	6	17 (15–20)	100 (86–120)	3.3	310	1600	97	23	3.1
St. 1	Caspian sprat	7	8.7 (8.2–9.5)	6.7 (5.4–7.7)	11	67	100	31	2.8	3.9
St. 2	Caspian sprat	7	9.1 (8.5–10)	6.7 (5.3–8.7)	9.4	120	280	38	4.4	5.1
St. 3	Caspian sprat	8	8.8 (8.0–9.5)	6.2 (4.8–8.3)	9.2	210	330	100	13	5.1
St. 4	Caspian sprat	8	9.1 (8.4–10)	6.8 (4.5–7.7)	8.1	130	260	37	7.5	5.1
St. 5	Caspian sprat	7	9.0 (8.5–9.5)	6.7 (5.3–8.5)	8.0	220	450	63	12	5.8

[a] Number of individuals homogenized.
[b] Body length.
[c] Body weight.
[d] Not measured since only muscle were available.

DDTs was the predominant contaminant with concentrations ranging from 3.1 to 560 μg/g, followed by PCBs (1.5–320 μg/g), HCHs (0.11–17 μg/g), CHLs (0.057–14 μg/g), HP-epox. (11–2600 ng/g), TCPMOH (7.3–9900 ng/g), TCPMe (1.0–950 ng/g), and HCB (1.2–160 ng/g). γ-HCH was below the detection limit (<0.01 μg/g on lipid weight) in all the samples analyzed. Elevated concentrations of DDTs and PCBs suggest the usage of these compounds in the past or recent years and also indicate the onerous status of contamination by OCs in Caspian seals. Although OC concentrations in male seals from Azerbaijan and Iran tended to be higher than those of corresponding females (in Azerbaijan, DDTs, HCHs, CHLs, HP-epox., TCPMOH: $p < 0.05$; in Iran, DDTs: $p < 0.05$; TCPMe: $p < 0.01$), the variation of OC residue levels with body length were unclear. Numbers of samples from other countries were too small to evaluate sex differences. Taking account of this result, data from all the male and female samples were combined for further discussion.

From a global point of view, DDT concentrations in Caspian seals were much higher than those reported for seals from Northern Europe (Roos et al., 1992; Blomkvist et al., 1992; Storr-Hansen and Spliid, 1993a), the Mediterranean Sea (Borrell et al., 1997; Cebrian et al., 1993), Japan (Nakata et al., 1998), and polar region (Fisk et al., 2002). DDTs residue levels found in Caspian seals were comparable to those in Baikal seals from Lake Baikal (Nakata et al., 1995) and California sea lions from west coast of USA (Kajiwara et al., 2001). This result suggests extensive usage of this insecticide around the Caspian Sea. Although the usage of DDT was banned in 1969 in the former Soviet Union, the contamination of sediments, soil and foodstuffs with DDTs were reported even recently, indicating possible illegal use of this pesticide until recently (Fedorov, 1999; De Mora et al., 2004).

PCB concentrations in Caspian seal were lower than those in other seals, including those that suffered from mass mortality in Northern Europe (Roos et al., 1992; Blomkvist et al., 1992; Storr-Hansen and Spliid, 1993a; Bernhoft and Skaare, 1994) and the Mediterranean Sea (Borrell et al., 1997; Cebrian et al., 1993), and comparable to those in Baikal seals from Lake Baikal (Nakata et al., 1995). These levels were also higher than those in seals from the Arctic (Fisk et al., 2002) and the Bering Sea (Krahn et al., 1997). In the former Soviet Union, technical PCB mixtures have been produced and used in both closed and open systems, such as capacitors, sealing paste additives and plasticizers, since the 1930s (Ivanov and Sandell, 1992).

OC residues in stranded seals from the five regions of Caspian Sea were compared to understand the spatial distribution of OCs (Fig. 1). OC concentrations in seals stranded in Azerbaijan were significantly higher than those from Iran. Some individuals with extremely high OCs had less fat contents in their blubber. For example, healthy Caspian seal normally contain >60% of fat in their blubber, but some animals found in this study contained only 20–30% (Table 1), indicating poor nutritive condition.

To elucidate whether this difference reflects the pollution of each location, OCs in fish samples of respective areas were also estimated in the present study (Table 2). Among the OCs analyzed, concentrations of DDTs (42–1600 ng/g) and PCBs (40–1800 ng/g) were the highest, followed by HCHs (6.8–170 ng/g), CHLs (2.2–36 ng/g) and HCB (1.4–14 ng/g). The predominance of DDT and PCB residue levels in Caspian fish of the present study was similar to those in other species of fish, sturgeons (Kajiwara et al., 2003), sediments (De Mora et al., 2004), and seals (this study; Hall et al., 1999; Kajiwara et al., 2002; Watanabe et al., 1999a) from the Caspian Sea. To understand the geographical variations in the accumulation characteristics, percentage compositions of OCs were calculated (Fig. 2). PCB/DDT ratio indicates that fishes from Kazakhstan had more PCBs than DDTs, and those from Azerbaijan and Turkmenistan contained comparable percentages of PCBs and DDTs. On the other hand, fishes from Iran retained DDTs predominantly. As for HCHs, β-HCH was the dominant isomer in fish samples, although fish from higher latitudes retained comparatively higher proportion of α-HCH (Fig. 2). Similar trend was also observed in Caspian sturgeons (Kajiwara et al., 2003). Composition of DDTs and CHLs were similar among locations. As stated above, the residue profile of OCs in fish showed a specific trend according to the sampling areas, reflecting the different usage patterns in respective countries. In contrast to fish, percentage compositions of OCs in Caspian seals collected from different countries closely resemble each other. This result suggests that Caspian seals were ubiquitously exposed to OCs throughout the Caspian Sea during their seasonal migration, and the OC residues are not reflecting local pollution sources.

In our previous study on Caspian seals, residue concentrations of OCs in epizootic animals were inversely related to blubber thickness, although no such correlation was found among seals collected in 1998 (Kajiwara et al., 2002). In the present investigation, significant negative correlation was

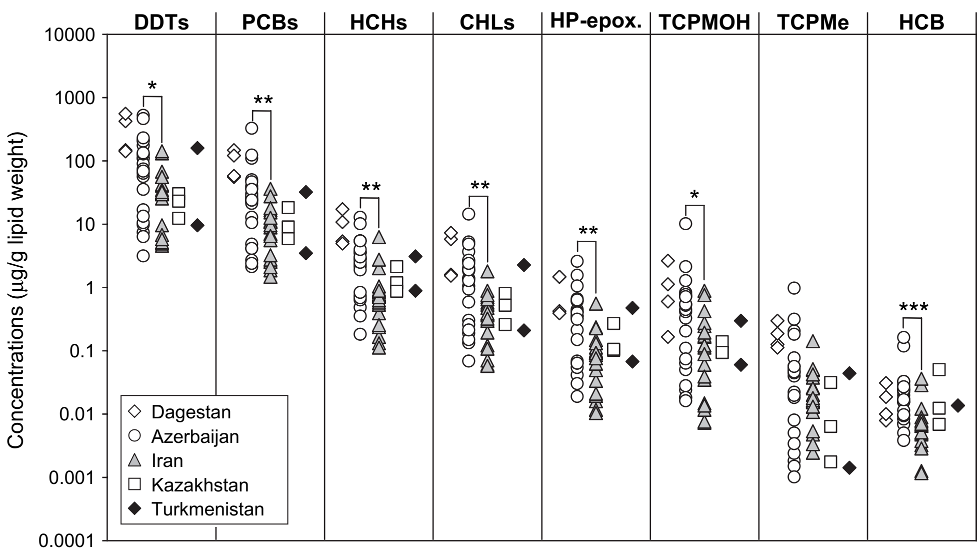

Fig. 1. Geographical distribution of organochlorine compounds in Caspian seals (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

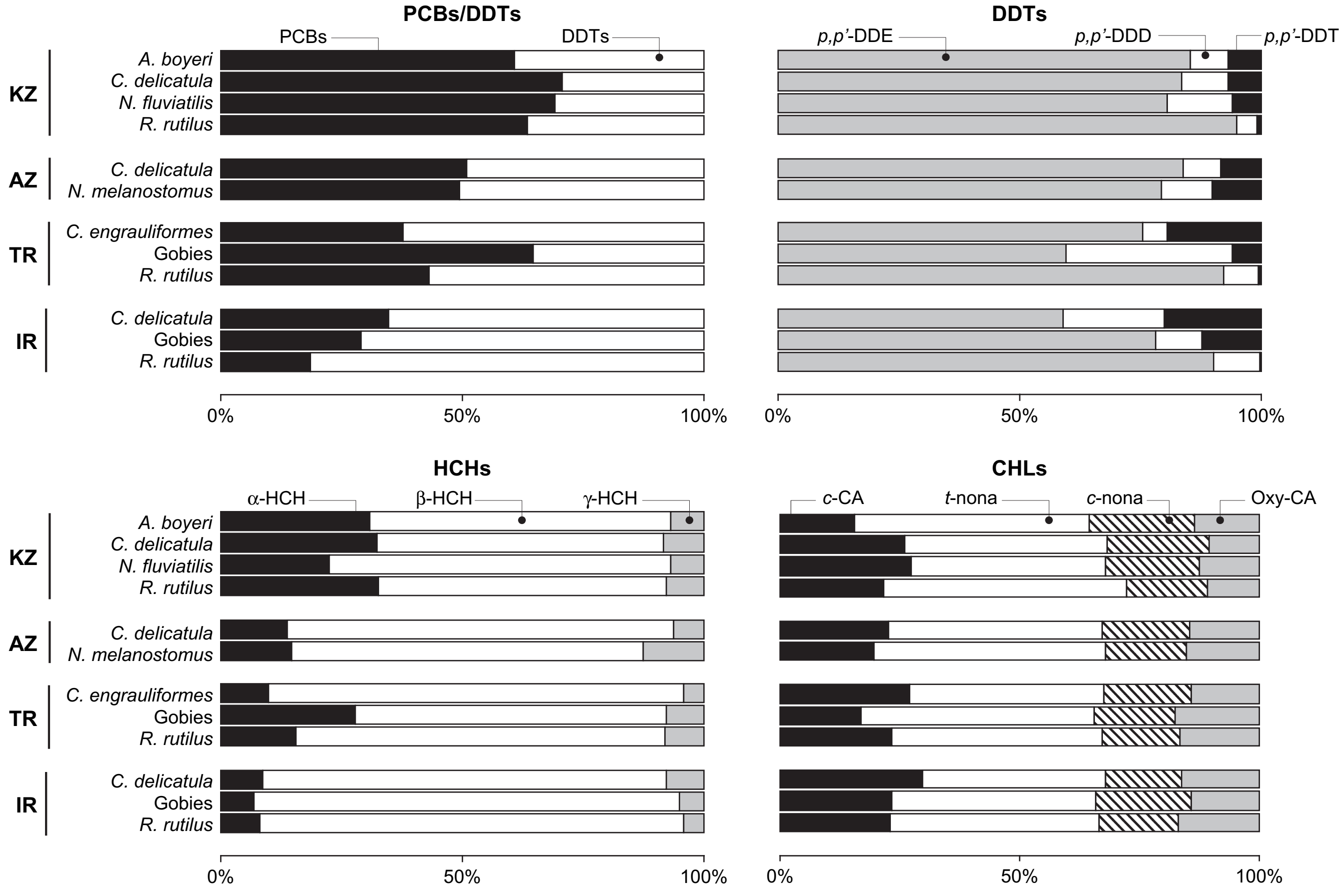

Fig. 2. Percentage compositions of DDT and CHL compounds and HCH isomers in bony fishes collected from Caspian Sea. KZ, Kazakhstan; AZ, Azerbaijan; TR, Turkmenistan; IR, Iran; c-CA, *cis*-chlordane; t-nona, *trans*-nonachlor; c-nona, *cis*-nonachlor; Oxy-CA, oxychlordane.

observed between OC levels and blubber thickness. When the blubber store decreased, the OC levels increased (Fig. 3). It suggests that when the animals mobilize their lipid stores, amount of blubber subsequently reduced and original amount of OCs were concentrated in the remaining lipid reserves. Fig. 4 shows the variation of blubber thickness with the data in the Caspian seals collected from northern Caspian Sea during a period of our previous investigations in 1993 and 1998. Apparently, seals collected from April to June had thinner blubber layer than those from September to December, indicating a clear seasonal variation. The blubber layer was significantly thicker in the seals collected from Iran that showed

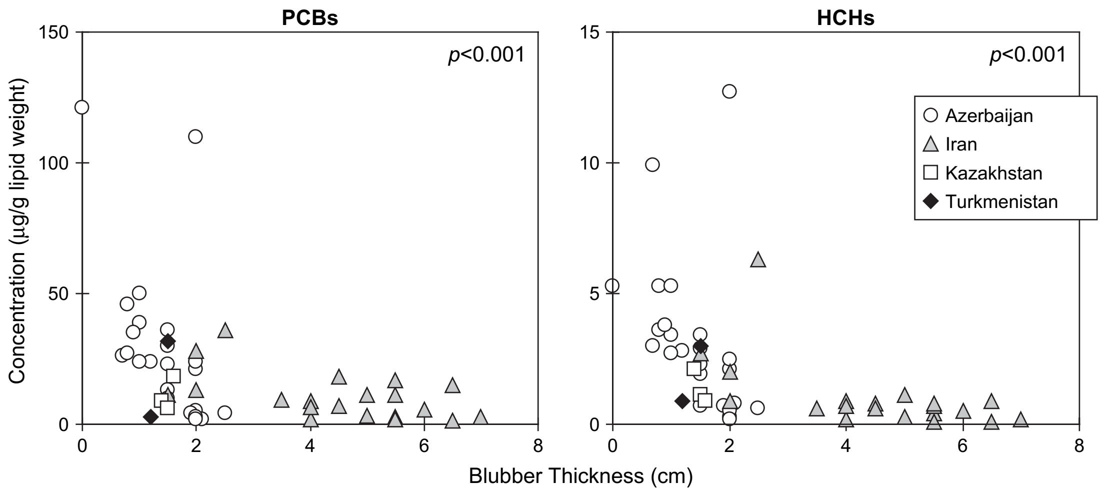

Fig. 3. Relationship between blubber thickness and OC levels in Caspian seals.

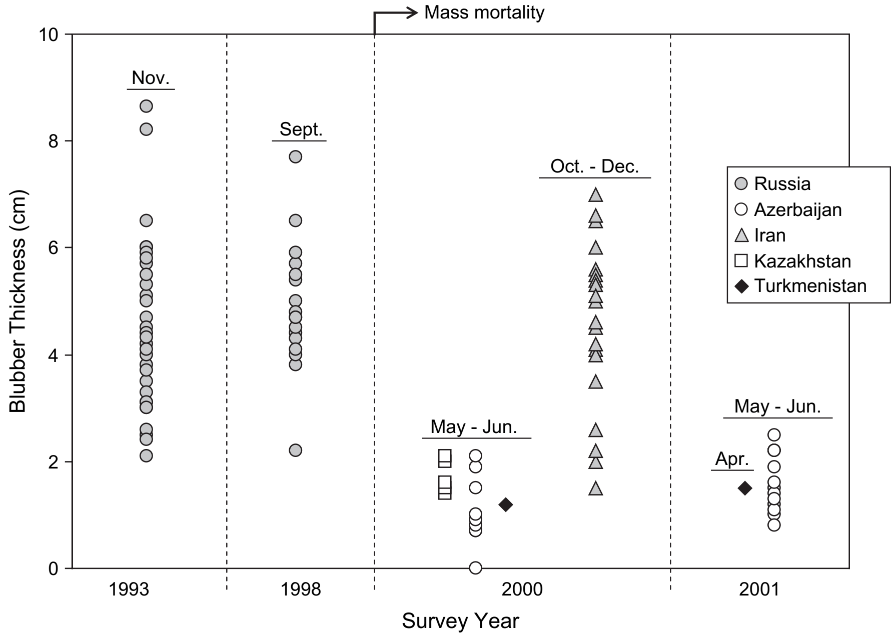

Fig. 4. Blubber thickness of Caspian seals collected during 1993, 1998, 2000 and 2001.

lower levels of OCs, than that of others found dead during mass mortality. Blubber weight of Caspian seals sometimes accounted for 50% of total body mass (Watanabe et al., 1999b). Therefore, a decline in blubber thickness indicates considerable utilization of fat and redistribution of OCs. Although migration pattern of Caspian seal is not fully understood, a northward migration in autumn and winter in preparation for breeding was observed (Popov, 1982). The season for parturition is from late January to the beginning of February. Pups are fed by their mothers for about a month on the ice of northern Caspian Sea. During a period of lactation and subsequent moulting, they are thought to be fasting (Bonner, 1984). The blubber store reduces due to the lactation and moulting, and OC levels in the organs and tissues become much higher than those before breeding season. In spring, they start to migrate southwards (Popov, 1982). This suggests that

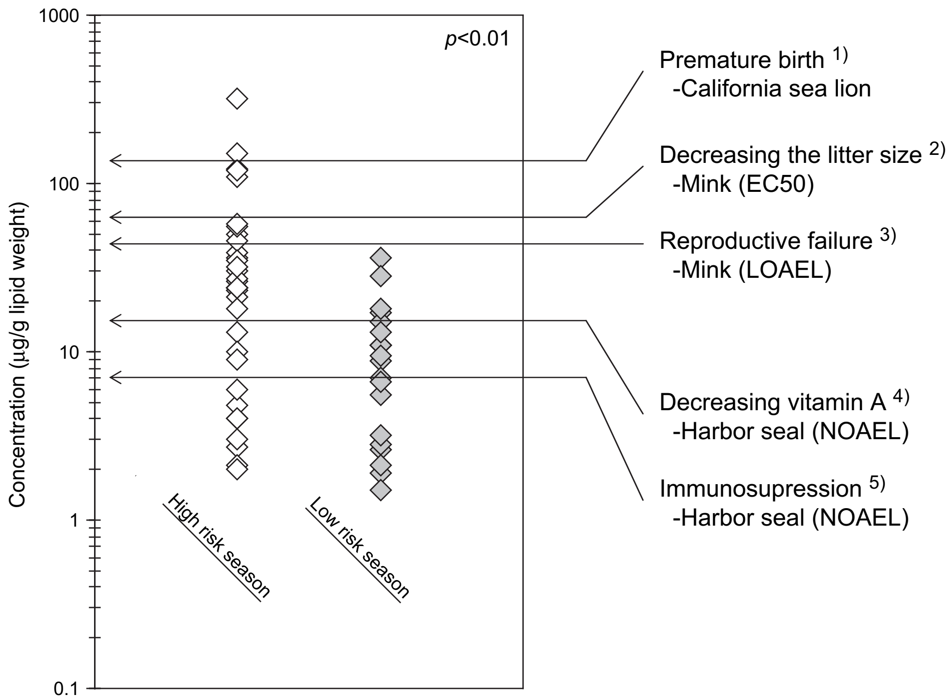

Fig. 5. Comparison of PCB concentrations in Caspian seals with the threshold levels of mammalian adverse effects. Data were cited from (1) De Long et al. (1973); (2) Kihlstrom et al. (1992); (3) Heaton et al. (1995); (4) Simms et al. (2000) and (5) De Swart et al. (1995).

Table 3
Concentrations and TEQs (pg/g lipid weight) of PCDD/Fs and DL-PCBs in the blubber of Caspian seals sampled in 1993 and 2000

Collected year	1993						2000												
Sample ID	93CS01	93CS04	93CS08	93CS20	93CS26	93CS38	KZPC-08	KZPC-09	KZPC-10	Baku 01	Baku 02	Baku 03	Baku 04	Baku 05	Baku 06	Baku 25	Baku 32	Baku E	T 1
Lipid (%)	87	92	86	90	93	87	93	83	83	41	75	22	84	85	71	85	78	80	81
Dioxins																			
2,3,7,8-T_4CDD	0.40	0.23	0.42	0.38	0.22	0.21	0.37	0.38	0.32	0.51	0.34	1.0	0.27	0.15	0.90	0.41	0.57	0.36	0.29
1,2,3,7,8-P_5CDD	0.89	0.78	0.66	1.0	1.3	0.51	2.4	1.8	1.6	4.5	0.74	11	1.1	0.64	3.1	0.89	1.2	0.79	0.38
1,2,3,4,7,8-H_6CDD	0.23	0.18	0.94	0.36	0.26	0.26	0.99	<0.1	0.58	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.54
1,2,3,6,7,8-H_6CDD	0.87	0.58	0.71	0.60	0.84	0.56	2.2	1.5	1.4	6.6	1.3	11	1.4	0.42	3.9	0.97	1.3	0.67	0.69
1,2,3,7,8,9-H_6CDD	0.17	<0.1	0.89	<0.1	0.30	<0.1	0.43	<0.1	0.37	<0.1	0.31	4.4	0.22	<0.1	1.1	0.42	<0.1	<0.1	0.41
1,2,3,4,6,7,8-H_7CDD	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0.76	5.9	0.67	<0.5	<0.5	0.53	<0.5	<0.5	<0.5
O_8CDD	1.4	2.4	2.4	0.79	1.2	2.2	0.96	1.1	1.3	3.8	2.3	5.3	0.73	0.52	2.0	2.2	2.6	2.5	1.2
Furans																			
2,3,7,8-T_4CDF	1.6	0.69	0.89	0.79	1.7	0.82	1.7	1.6	0.48	1.2	0.49	2.0	1.0	0.33	1.9	1.3	1.1	0.95	0.81
1,2,3,7,8-P_5CDF	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
2,3,4,7,8-P_5CDF	1.6	0.54	1.1	0.84	1.2	0.34	3.8	2.0	2.1	24	2.4	23	1.7	0.49	3.6	2.6	4.9	2.1	0.62
1,2,3,4,7,8-H_6CDF	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
1,2,3,6,7,8-H_6CDF	0.40	0.40	1.1	0.78	0.57	0.43	1.1	1.1	0.71	1.9	0.68	4.0	1.2	0.29	2.3	0.86	1.0	0.52	0.97
1,2,3,7,8,9-H_6CDF	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
2,3,4,6,7,8-H_6CDF	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
1,2,3,4,6,7,8-H_7CDF	<0.1	<0.1	<0.1	0.51	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.28	1.5	0.33	<0.1	0.37	0.25	<0.1	0.64	<0.1
1,2,3,4,7,8,9-H_7CDF	<0.1	<0.1	1.1	1.6	<0.1	<0.1	<0.1	<0.1	0.44	1.9	<0.1	1.1	0.16	<0.1	0.81	0.26	<0.1	1.4	<0.1
O_8CDF	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.4	<0.1	<0.1	<0.1	<0.1
non-ortho DL-PCBs																			
3,3′,4,4′-T_4CB (77)	31	29	27	23	43	22	10	20	14	43	10	59	18	8.1	30	25	19	20	13
3,4,4′,5-T_4CB (81)	1.3	1.4	1.2	1.1	1.3	0.89	0.94	1.9	0.74	3.4	0.53	5.4	1.4	0.34	2.0	1.9	2.1	1.2	0.72
3,3′,4,4′,5-P_5CB (126)	81	60	39	59	97	44	94	100	200	240	49	350	53	26	190	68	81	70	31
3,3′,4,4′,5,5′-H_6CB (169)	1.8	30	18	29	23	19	77	83	67	250	40	410	66	14	270	52	99	59	15
mono-ortho DL-PCBs																			
2,3,3′,4,4′-P_5CB (105)	9200	8400	3900	6900	9900	6100	17,000	17,000	19,000	36,000	6800	65,000	7000	4400	26,000	17,000	13,000	9900	6100
2,3,4,4′,5-P_5CB (114)	490	470	280	390	520	750	700	950	830	1300	300	2200	320	200	1400	630	490	450	210
2,3′,4,4′,5-P_5CB (118)	43,000	37,000	19,000	32,000	45,000	24,000	71,000	68,000	89,000	230,000	39,000	380,000	35,000	19,000	130,000	76,000	71,000	56,000	24,000
2′,3,4,4′,5-P_5CB (123)	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
2,3,3′,4,4′,5-H_6CB (156)	19,000	14,000	6200	13,000	14,000	10,000	28,000	19,000	54,000	270,000	24,000	300,000	12,000	5700	68,000	33,000	50,000	33,000	7900
2,3,3′,4,4′,5′-H_6CB (157)	6700	4200	2100	5500	4400	3800	9800	5800	20,000	140,000	11,000	130,000	4400	1700	21,000	13,000	19,000	12,000	2300
2,3′,4,4′,5,5′-H_6CB (167)	730	460	280	570	900	1600	600	680	690	3700	680	10,000	490	190	960	1400	750	700	310
2,3,3′,4,4′,5,5′-H_7CB (189)	2700	1700	1500	2700	1400	2400	2800	1700	7400	140,000	7000	140,000	2100	700	9900	9100	16,000	9900	730
Total PCDDs	4.0	4.1	6.0	3.1	4.1	3.8	7.4	4.7	5.7	15	5.7	38	4.5	1.7	11	5.4	5.7	4.4	3.5
Total PCDFs	3.7	1.6	4.1	4.5	3.4	1.6	6.5	4.7	3.8	29	3.8	31	4.5	1.1	10	5.2	7.0	5.5	2.4
Total PCDD/Fs	7.7	5.8	10	7.7	7.5	5.3	14	9.5	9.4	44	9.6	69	8.9	2.8	21	11	13	9.9	5.9
PCDDs-TEQs	1.4	1.1	1.3	1.5	1.7	0.81	3.2	2.3	2.2	5.7	1.2	13	1.6	0.82	4.5	1.4	1.9	1.2	0.84
PCDFs-TEQs	1.0	0.38	0.74	0.60	0.80	0.30	2.2	1.3	1.2	12	1.3	12	1.1	0.30	2.2	1.5	2.7	1.2	0.49
PCDD/Fs-TEQs	2.4	1.5	2.1	2.1	2.5	1.1	5.3	3.6	3.4	18	2.6	25	2.7	1.1	6.7	2.9	4.6	2.4	1.3
Total non-ortho DL-PCBs	120	120	85	110	160	86	180	210	280	540	99	830	140	48	500	150	200	150	59
Total mono-ortho DL-PCBs	82,000	66,000	33,000	61,000	76,000	49,000	130,000	110,000	190,000	820,000	88,000	1,000,000	61,000	32,000	250,000	150,000	170,000	120,000	42,000
Total DL-PCBs	82,000	66,000	33,000	61,000	76,000	49,000	130,000	110,000	190,000	820,000	89,000	1,000,000	61,000	32,000	250,000	150,000	170,000	120,000	42,000
non-ortho DL-PCBs-TEQs	8.1	6.3	4.1	6.2	9.9	4.6	10	11	21	26	5.3	39	6.0	2.7	22	7.3	9.1	7.6	3.2
mono-ortho DL-PCBs-TEQs	19	14	6.7	14	15	11	28	22	49	250	23	280	13	6.2	61	34	45	30	8.3
Total DL-PCBs-TEQs	27	20	11	20	25	15	38	33	70	270	28	310	19	8.9	83	41	54	38	12
Total TEQs	29	22	13	22	27	16	44	36	73	290	31	340	21	10	90	44	58	40	13

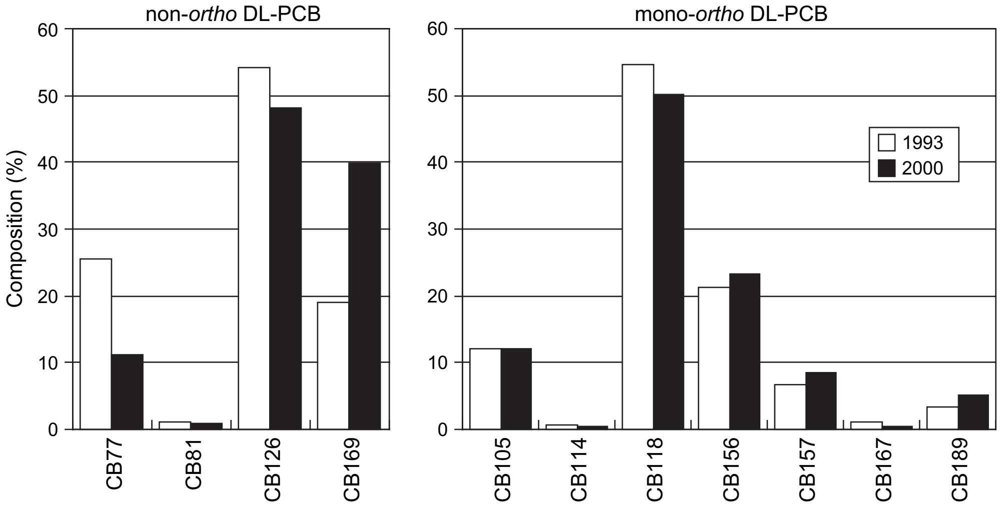

Fig. 6. Comparison of the compositions of non- and mono-*ortho* DL-PCBs in Caspian seals collected in 1993 and 2000.

Caspian seals might face higher risk due to high concentration of OCs in their bodies during spring to summer. Considering these observations, we suggest that Caspian seals may have ''high risk season'' from spring to summer, and ''low risk season'' from autumn to winter. Except for TCPMe, OC levels in seals in high risk season were significantly higher than those in low risk season (PCBs in Fig. 5: $p < 0.005$; DDTs and TCPMOH: $p < 0.05$; HCHs, CHLs, HP-epox. and HCB: $p < 0.001$). In addition, PCB concentrations in diseased Caspian seals in high risk season exceeded the levels associated with mammalian adverse effects (De Long et al., 1973; Kihlstrom et al., 1992; Heaton et al., 1995; Simms et al., 2000; De Swart et al., 1995) (Fig. 5). These results suggest that elevated levels of exposure to OCs might be linked to immunosuppression and seals might be susceptible to virus infection.

3.2. Contamination status of dioxin related compounds

Total concentrations of PCDDs/DFs and DL-PCBs showed a range of 33,000−82,000 pg/g and 32,000−1,000,000 pg/g in

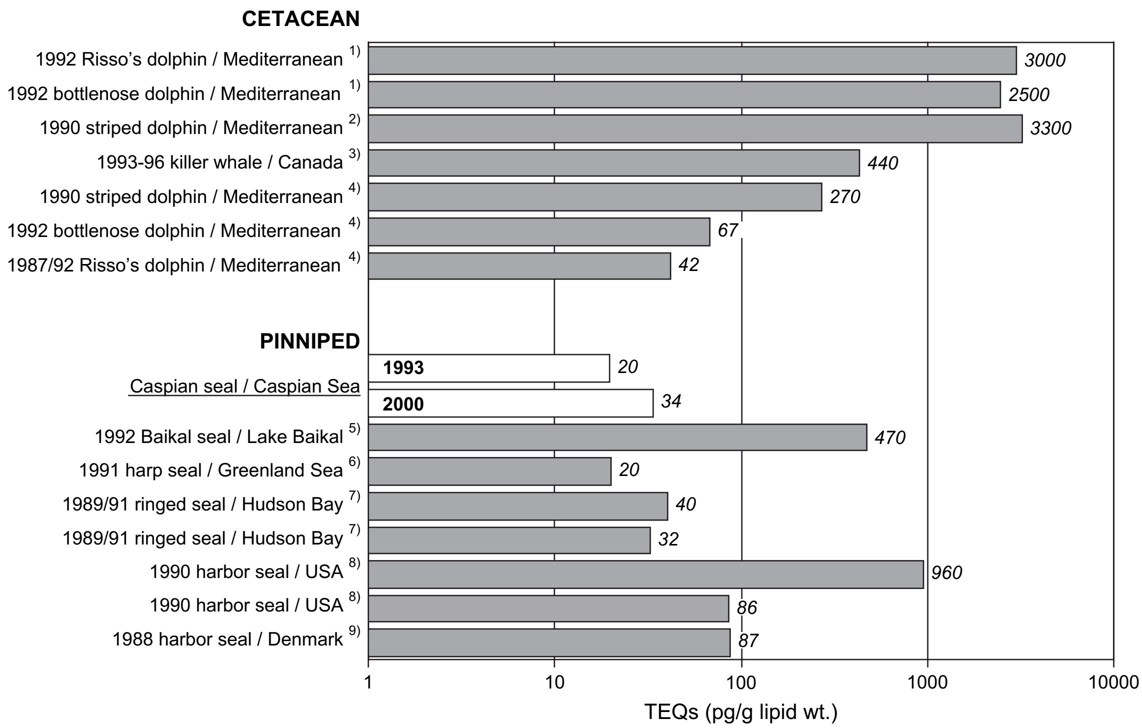

Fig. 7. Comparison of TEQ concentrations in Caspian seals with those in other aquatic mammals. Data were cited from (1) Corsolini et al. (1995); (2) Kannan et al. (1993); (3) Ross et al. (2000); (4) Jimenez et al. (2000); (5) Iwata et al. (2004); (6) Oehme et al. (1994); (7) Muir et al. (1995); (8) Hong et al. (1996) and (9) Storr-Hansen and Spliid (1993b).

the blubber of Caspian seals collected in 1993 (non-epizootic) and 2000 (diseased animals), respectively (Table 3). Congener-specific analysis of PCDDs/DFs showed that only certain congeners occurred frequently and most others were below the limits of detection. For example, 1,2,3,7,8-P_5CDF, 1,2,3,4,7,8-H_6CDF, 1,2,3,7,8,9-H_6CDF, and 2,3,4,6,7,8-H_6CDF were not detected in all the seal samples and 1,2,3,4,7,8-H_6CDD, H_7CDD/DFs, and OCDF were not detected in most of the animals analyzed. In both time periods, therefore, mono-*ortho* DL-PCBs were the predominant contaminants contributing more than 95% of the total dioxins and related compounds measured, followed by non-*ortho* DL-PCBs, and PCDDs/DFs. Concentrations of total DL-PCBs in diseased animals collected in 2000 were significantly higher than those in seals collected in 1993. As mentioned earlier, fat reserves in diseased Caspian seals were mobilized and the contaminants got concentrated in the remaining blubber (Fig. 3). Similar variation profiles were also found for DL-PCBs as well as PCDDs/DFs. Therefore, these results indicate that input of dioxin related compounds in Caspian ecosystem did not occur recently.

Among the mono-*ortho* DL-PCBs, the residue level of CB118 was the highest, and CB123 was a less accumulated congener. As shown in Fig. 6, the proportion of CB118, which is largely present in PCB preparations (Ivanov and Sandell, 1992), were low in diseased seals collected in 2000. In the case of non-*ortho* DL-PCBs, contribution by CB77 in the epizootic seals was less than those in animals collected in 1993, whereas the proportion of CB169 was higher (Fig. 6). Given that PCB preparations contain larger proportion of CB77 than CB126 and CB169 (Kannan et al., 1987), these results suggest a declining trend of PCB contamination in the Caspian Sea.

To estimate the toxic potential of dioxins and related compounds, 2,3,7,8-TCDD toxic equivalents (TEQs) in the Caspian seals were calculated from mammalian toxic equivalent factors (TEFs) developed by WHO (Van den Berg et al., 1998) (Table 3). Total TEQ concentrations in seals that died in the 2000 epizootic (10–340 pg TEQ/g) were significantly higher than those in healthy animals collected in 1993 (13–29 pg TEQ/g). TEQ levels in two individuals among 19 animals analyzed exceeded the level associated with immune dysfunction in harbor seals demonstrated from a 2-year semi-field study (209 pg TEQ/g, Ross et al., 1995). The percentage contribution of TEQs derived from DL-PCBs to total TEQs was 83–95% in Caspian seals, indicating DL-PCBs are significant contaminants for use in risk assessment when compared to PCDDs/DFs in this species. Among non- and mono-*ortho* DL-PCBs, TEQs of either CB126 or CB156 had the highest contribution in this study, followed by CB118, CB105, CB169 and CB77. When compared to other seals, lowest percentage of non-*ortho* DL-PCBs, but higher proportion of mono-*ortho* congener CB156 in Caspian seals was observed. This pattern was rather similar to those in many cetaceans (Kannan et al., 1993; Corsolini et al., 1995).

Individuals of both epizootic Caspian seals in 2000 and non-epizootic ones in 1993 accumulated apparently lower TEQ levels than those observed in Baikal seals from the Lake Baikal (Iwata et al., 2004) and harbor seals from the North Sea (Storr-Hansen and Spliid, 1993b), where mass mortalities took place in the past (Fig. 7). TEQs in Caspian seals were comparable to those in seals inhabiting subpolar regions (Oehme et al., 1994; Muir et al., 1995). Considering all these facts, toxic effects of dioxins and related compounds in Caspian seals can be considered as a deal less and are unlikely to be linked to mass mortality.

In conclusion, toxicological studies are required on Caspian seals in the high risk season considering the fact that no information is available on the toxic effects of environmental contaminants in these animals. Although total TEQs in Caspian seals were relatively low even in diseased animals, present status of PCBs and OC pesticides contamination found in these seals might pose a risk of immunosuppression. Therefore, even if the vital cause of mass mortality is still unclear, toxic effects of persistent OCs cannot be ruled out.

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