Chemical/biological analyses of harbour porpoise samples to identify contaminants in the food chains

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Report number C140/07



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Publication Date: December 15th 2007

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A_4_3_2-V4

Contents

Summary

Polychlorinated biphenyls (PCBs 31, 28, 52, 49, 47, 44, 95+66, 101, 56, 97, 87, 85, 110, 151, 149, 118, 153, 141, 105, 137, 138, 187, 202, 128, 156, 180, 170, 194, 206), brominated flame retardants (PBDEs 28, 47, 49, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 183, 190, 206, 207, 208 and 209, α-, β-, γ- isomers of HBCD and TBBP-A and me-TBBP-A), perfluorinated compounds (PFOA, PFOS, PFHxA, PFNA, PFUnA, PFBA, PFBS, PFDoA, PFDcA, PFOSA and PFHxS), organotin compounds (tributyltin, dibutyltin, monobutyltin, triphenyltin, diphenyltin, monophenyltin) and sample characteristics such as dry weight, protein and fat content were analysed in twelve blubber and/or twelve liver samples of harbour porpoises found stranded in 2006 on the Dutch coast. In this document, information on sample origin is summarized, applied sample processing is described, used analytical methods are specified and measured concentrations are listed. In addition, graphical comparison of compound distribution among the samples is provided.

1. Introduction

One of the main objectives of the "Koploper" project "Identificatie van mogelijke probleemstoffen in voedselketens en toppredatoren" is to identify possible causes of the increasing number of harbour porpoises that are stranded on the Dutch coast. In order to find out whether chemical contamination could cause the increased stranding, Deltares has contracted IMARES to analyse main organohalogenated contaminants in the harbour porpoises found stranded during year 2006. The samples of harbour porpoise (liver and blubber) were collected by IMARES, Texel within the LNV project "Bruinvisstrandingen in Nederland in 2006 – Achtergronden, leeftijdsverdeling, sexratio, voedselkeuze en mogelijke oorzaken" (Leopold & Camphuysen, 2006). From 64 harbour porpoises examined in that project, 12 animals were selected for the chemical analyses. The selection was based on the following parameters: juvenile males, fresh material (no animals in a state of decomposition) and fair up to well fed animals. 12 selected porpoises originated from the coast of Texel (4), North Holland (3) and Zeeland (5).
This document provides information on sample origin, sample processing and analyses and reports concentrations of PCBs, flame retardants (PBDEs, TBBP-A, me-TBBP-A, α, β, γ-HBCD), perfluorinated compounds (PFOS, PFOA, PFHxA, PFNA, PFUnA, PFBA, PFBS, PFDoA, PFDcA, PFOSA, PFHxS) and organotin compounds (TBT, DBT, MBT, TPhT, DPhT, MPhT) found in the selected samples of stranded harbour porpoises. Sample characteristics, such as dry weight, fat and protein content are provided as well.

2. Materials and Methods

Sample description, delivery and storage

Twelve liver and twelve blubber samples of twelve stranded harbour porpoises were obtained from Drs. S.M.J.M. Brasseur of IMARES, Texel. The samples of harbour porpoise (liver and blubber) were collected by IMARES, Texel within the LNV project "Bruinvisstrandingen in Nederland in 2006 – Achtergronden, leeftijdsverdeling, sexratio, voedselkeuze en mogelijke oorzaken" (Leopold & Camphuysen, 2006). A typical liver and blubber sample received are visualized in Figs. 2.1 and 2.2, respectively. Overview of the samples origin and background information are given in Table 2.1. Please note that information provided in the Table 2.1. was extracted from the report "Bruinvisstrandingen in Nederland in 2006 – Achtergronden, leeftijdsverdeling, sexratio, voedselkeuze en mogelijke oorzaken" (Leopold & Camphuysen, 2006).
The samples were delivered to IMARES' laboratory in IJmuiden on September 25, 2007. After the delivery, unique LIMS numbers were assigned to each of the samples and the samples were stored in the refrigerator at -20°C till their analyses.

Fig. 2.1. Liver sample of a stranded harbour porpoise

Fig. 2.2. Blubber sample of a stranded harbour porpoise

Table 2.1 Background information about the 12 porpoise samples

Sample code	LIMS-nr.	Sample type	Age	Gender	Length (cm)	Weight (kg)	Date running ashore	Location	Condition cadaver
A07/041 TX40	2007/0894 2007/0895	Liver Blubber	Juvenile	Male	111	21	11-3-2006	Texel dijk Ceres	fresh
A07/014 TX58	2007/0896 2007/0897	Blubber Liver	Juvenile	Male	105	17	28-3-2006	Ouddorp	very fresh
A07/033 TX5	2007/0898 2007/0899	Blubber Liver	Juvenile	Male	101	n.d.	6-3-2006	Groote Keeten	fresh
A07/034 TX14	2007/0900 2007/0901	Blubber Liver	Juvenile	Male	103	20	5-3-2006	Texel p29	fresh
A07/016 TX61	2007/0902 2007/0903	Blubber Liver	Juvenile	Male	87	11	21-6-2006	Vlissingen	very fresh
A07/027 TX10	2007/0904 2007/0905	Blubber Liver	Juvenile	Male	111	19	1-3-2006	St Maartenszee p17	fresh
A07/031 TX26	2007/0906 2007/0907	Blubber Liver	Juvenile	Male	103	16	28-6-2006	Texel p19.5	fresh
A07/005 TX13	2007/0908 2007/0909	Blubber Liver	Juvenile	Male	116	18	1-9-2006	Texel p28	very fresh
A07/026 TX41	2007/0910 2007/0911	Blubber Liver	Juvenile	Male	99	14	28-3-2006	Kerkwerve	very fresh
A07/040 TX46	2007/0912 2007/0913	Blubber Liver	Juvenile	Male	101	25	15-3-2006	Burgh	fresh
A07/008 TX33	2007/0914 2007/0915	Blubber Liver	Juvenile	Male	107	21	2-3-2006	Dishoek	very fresh
A07/039 TX57	2007/0916 2007/0917	Blubber Liver	Juvenile	Male	102	17	29-4-2006	Bergen aan Zee	fresh

Pretreatment of the samples

Both liver and blubber samples were homogenized before the analyses. Homogenization took place on September 27, 2007. Liver samples were homogenized by blender as shown in Fig. 2.3. Blubber samples were pealed before the homogenization; the outside part of the sample, skin, and the inside part of the sample, bloody part, was removed. Homogenization of the blubber samples could not be performed by the blender, because material was too supple. Blubber samples were therefore cut into small pieces (see Fig. 2.4) and subsamples were taken by random selection.

Fig. 2.3. Homogenization of liver samples

Fig. 2.4. Homogenization of blubber samples

Dry weight determination

The dry weight content was determined for all 24 (12 liver and 12 blubber) samples. The determination was performed gravimetrically following ISO 17025:2005 accredited method. Every sample was weighed before and after drying in the oven at temperature of 105 ± 5°C for 3 hours. Each sample was determined in duplicate and reported values are means of two replicates.

Fat content determination

Fat content was determined in the blubber samples. Two values are reported in this report for each sample, because two methods were used. First, total fat content was determined by Bligh & Dyer (B&D) method. Second, extractable lipids were determined after Soxhlet extraction step in the PCB determination procedure.
Bligh & Dyer method used is ISO 17025:2005 accredited. The samples were three times extracted by solvent mixture of chloroform, methanol and demineralized water. The fat content was determined gravimetrically after evaporation of the solvents. One should remember that fat determination by B&D method is performed in the individual portion of the total sample, or in other words, parallel to other determinations.
Extractable lipids were determined after Soxhlet extraction of blubber sample by pentane/dichloromethane (1:1). Weighed portion of the extract was transferred to the Petri dish and amount of fat was determined gravimetrically after evaporation of the solvent. By this method, extractable amount of fat is determined in the same sample portion in which PCB concentration is determined.

Protein content determination

Protein content was determined in the liver samples. The analyses were subcontracted to Analytico food – Eurofins. The determination was performed according to NEN-EN-ISO 8968 2004 which is based on Kjeldahl method. The method consists of heating a substance with sulfuric acid which decomposes the organic nitrogen present to ammonium sulfate. The solution is then distilled with sodium hydroxide (added in small quantities) which converts the ammonium salt to ammonia. The amount of ammonia present (hence the amount of nitrogen present in the sample) is determined by back titration. The end of the condenser is dipped into a solution of hydrochloric acid or sulfuric acid of precisely known concentration. The method used by Analytico is ISO 17025:2005 accredited.

Determination of PCBs

Concentrations of 29 PCBs (CBs 31, 28, 52, 49, 47, 44, 95+66, 101, 56, 97, 87, 85, 110, 151, 149, 118, 153, 141, 105, 137, 138, 187, 202, 128, 156, 180, 170, 194, 206) were determined in twelve blubber samples. The method used is ISO 17025:2005 accredited and is based on GC-ECD determination on two column system after Soxhlet extraction and clean-up on alumina and silica gel column. More detailed description of the method is as follow. Sample was mixed with sodium sulphate and Soxhlet extracted for 6.5 hours with 140 ml pentane/dichloromethane (1:1). To remove the fat, clean-up and fractionation was performed on 15 g alumina · 8% H_2O column. The PCB fraction was eluted with 100 mL n-pentane. After concentration of the eluate by Rotavap, the concentrate was fractionated on a silica column (1.8 g silica · 1.5% H_2O) to separate PCBs from other co-extracted compounds. PCBs were collected in the first fraction by elution with 11 mL of iso-octane. The final extract was transferred to an autosampler vial and injected on GC-ECD. Final determination was performed by gas chromatography (GC) with electron capture detection (ECD) using two column system: Cp-Sil 8 (50 m long with internal diameter of 0.15 mm and film thickness of 0.20 µm) and Cp-Sil 19 (50 m long with internal diameter of 0.15 mm and film thickness of 0.20 µm). PCB concentrations in each sample were calculated using data from both columns. Reported concentration was always the one with lower value.

Determination of brominated flame retardants

Concentrations of 20 PBDE congeners (BDE 28, 47, 49, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 183, 190, 206, 207, 208 and 209), α, β, γ isomers of HBCD and TBBP-A and me-TBBP-A were determined in twelve blubber samples. The method used is ISO 17025:2005 accredited and its brief description is as follow. Sample mixed with sodium sulphate was Soxhlet extracted for 6.5 hours with hexane/acetone (3:1). To ensure extraction of TBBP-A, diluted sulphuric acid was added to concentrated extract and the organic phase was recovered by pipette. After drying the organic phase by filtration through the sodium sulphate and concentration to 2 mL, the extract was cleaned by gel permeation chromatography. PL gel (600 mm x 25 mm i.d., 10 μm particle size, 50 A pore size) column was used and compounds were eluted with dichloromethane at 10 ml/min. Fraction between 16.3 and 24 min was collected. To ensure that all fat was removed, the cleaned extract was further treated with concentrated sulphuric acid. 1 mL of concentrated sulphuric acid was added to the extract and organic phase was recovered by pippete after addition of 1 mL of iso-octane. Finally, the extract concentrated to 2 mL was fractioned on 1.5 g SiO_2 . 1.5% H_2O column . The fraction eluted with 11 mL of iso-octane and 29 mL of diethylether/iso-octane (85:15, v/v) was collected. After addition of 0.5 mL of toluene, the eluate was concentrated to 1 mL and transfer to an autosampler vial. Determination of PBDEs and me-TBBP-A was performed by GC-ECNI-MS in SIM mode and determination of α, β, γ HBCD isomers and TBBP-A by HPLC-ESI-MS. PBDEs with up to 8 Br atoms in the molecule were determined using Cp-Sil 8 CB column (50 m long with internal diameter of 0.25 mm and film thickness of 0.25 μm) while PBDEs with 9 and 10 Br atoms in the molecule, i.e. BDEs 206, 207, 208 and 209, were determined using shorter DB-5 column (15 m long with internal diameter of 0.25 mm and film thickness of 0.25 μm).

Determination of perfluorinated compounds

Concentrations of 11 perfluorinated compounds (PFOA, PFOS, PFHxA, PFNA, PFUnA, PFBA, PFBS, PFDoA, PFDcA, PFOSA and PFHxS) were determined in twelve liver samples. The analyses were performed following a validated procedure. Samples were extracted 3 times by shaking with ecetonitrile for ½ hour. After each shaking, acetontrile was recovered by centrifugation. Combined extracts were then concentrated to ca. 1 mL and cleaned by addition of active carbon to the extract. Acetonitrile was then separated from the carbon by centrifugation and transferred by pipette to a autosampler vial. Final determination was performed by HPLC-ESI-MS using ion-trap instrument.

Determination of organotin compounds

Concentrations of 6 organotin compounds (tributyltin (TBT), dibutyltin (DBT), monobutyltin (MBT), triphenyltin (TPhT), diphenyltin (DPhT), monophenyltin (MPhT)) were determined in twelve liver samples. For this purpose, a new method based on the method of RIKZ, Haren was implemented in the laboratory. In this method, extraction and derivatization was performed simultaneously. First, 15 mL of methanol, 1.5 mL of acetic acid and 7 mL of hexane was added to the freeze-dried sample and the mixture was shaken for at least 5 min. After addition of 3 mL of 4M sodium acetate, the ionic organotin compounds were alkylated by continuous addition of sodium tetraethylborate (4 mL in ca. 24 min) and vigorous stirring. The tetra-alkylated compounds migrated from water to hexane phase. After addition of 5 mL of 10M NaOH and milli-Q water, the organic phase was recovered by pipette from centrifuged mixture. Clean-up was performed on mixed column containing 1 g of C18 at the bottom and 8 g of Al_2O_3 at the top. The derivatized organotin compounds were eluted with 10 mL of hexane. After concentration of the sample in iso-octane, the compounds were analyzed with GC-EI-MS in the SIM mode using HT-8 column (60 m long with internal diameter of 0.25 mm and film thickness of 0.25 μm).

3. Results

Dry weight, fat and protein content

Results of the dry weight, fat and protein content are given in Table 3.1. The values obtained are similar for all samples. Only exception is blubber sample (2007/0908) for sample A07/005 TX13. The total fat (B&D) and dry weight content is approximately half of the values for other samples and extractable fat is significantly higher than total fat. This deviation is due to very lean sample. Not enough fat was present in the sample. Consequently, inside part, i.e. bloody part, of the sample was not removed and was taken into analyses. This part however contains significantly lower amount of fat and higher amount of water. Since measurements of total fat and dry weight was performed at the end, only pieces from the bloody part remained available. Therefore, these values should not be taken as representative. If normalization on fat basis should be done, it is advised to use extractable fat.

Table 3.1. Results (%) of dry weight and protein for 12 liver samples and of dry weight, total and extractable fat for 12 blubber samples.

LIMS No.	Sample code	Matrix	Dry weight* %	Protein content %	Dry weight* %	Total fat (B&D) %	Extractable fat %
2007/0894	A07/041 TX40	Liver	26,1	22,0	-	-	-
2007/0895		Blubber	-	-	89,4	93,0	91,1*
2007/0896	A07/014 TX58	Blubber	-	-	72,6	75,7	83,4
2007/0897		Liver	25,8	21,8	-	-	-
2007/0898	A07/033 TX5	Blubber	-	-	95,3	96,0	95,9
2007/0899		Liver	30,3	23,7	-	-	-
2007/0900	A07/034 TX14	Blubber	-	-	91,8	92,6	89,8
2007/0901		Liver	26,6	21,9	-	-	-
2007/0902	A07/016 TX61	Blubber	-	-	91,0	92,5	88,5
2007/0903		Liver	26,6	22,3	-	-	-
2007/0904	A07/027 TX10	Blubber	-	-	90,8	93,5	85,4
2007/0905		Liver	25,6	21,6	-	-	-
2007/0906	A07/031 TX26	Blubber			84,2	91,7	87,2
2007/0907		Liver	28,3	23,4	-	-	-
2007/0908	A07/005 TX13	Blubber	-	-	49,4	35,3	70,8
2007/0909		Liver	29,0	24,3	-	-	-
2007/0910	A07/026 TX41	Blubber	-	-	88,9	89,0	87,7
2007/0911		Liver	25,1	21,5	-	-	-
2007/0912	A07/040 TX46	Blubber	-	-	98,0	98,3	99,6
2007/0913		Liver	26,4	21,8	-	-	-
2007/0914	A07/008 TX33	Blubber	-	-	96,6	97,9	98,0
2007/0915		Liver	29,4	21,8	-	-	-
2007/0916	A07/039 TX57	Blubber	-	-	94,3	96,8	92,6
2007/0917		Liver	29,7	26,3	-	-	-

* measured in duplicate; reported value is average of 2 replicates

PCBs

Concentrations of PCB congeners measured in blubber samples are given in Table 3.2. and graphical visualization is shown in Fig. 3.1. Although analyses of only 7 indicator PCBs were contracted, 29 PCB congeners were performed to provide more comprehensive characterization of PCB pattern in the harbour porpoise samples. Comparison of the PCB patterns among the harbour porpoise samples can provide information on uniformity of the contamination source and comparison between PCB pattern in harbour porpoises and their preys can be a useful tool to trace the uptake route.

Table 3.2. Concentrations of PCBs (µg/kg fresh weight) measured in 12 blubber samples.

LIMS No.	Sample code	CB-31 µg/kg	CB-28 µg/kg	CB-52 µg/kg	CB-49 µg/kg	CB-47 µg/kg	CB-44 µg/kg	CB-95+66 µg/kg
2007/0895	A07/041 TX40	<10	<14	280	110	67	39	nb
2007/0896	A07/014 TX58	<8,9	7,9	320	120	81	35	nb
2007/0898	A07/033 TX5	<21	<29	500	94	63	23	nb
2007/0900	A07/034 TX14	<99	<14	210	68	42	18	nb
2007/0902	A07/016 TX61	<20	19	1600	360	310	120	nb
2007/0904	A07/027 TX10	<9,8	<14	460	170	98	53	nb
2007/0906	A07/031 TX26	<20	<28	560	96	96	27	nb
2007/0908	A07/005 TX13	<16	56	3000	840	710	260	nb
2007/0910	A07/026 TX41	<20	<27	250	68	52	20	nb
2007/0912	A07/040 TX46	<5,9	6,1	84	49	23	15	nb
2007/0914	A07/008 TX33	<11	<15	440	190	97	58	nb
2007/0916	A07/039 TX57	<5,0	39	480	200	110	85	nb

Table 3.2. continuation

LIMS No.	Sample code	CB-101 µg/kg	CB-56 µg/kg	CB-97 µg/kg	CB-87 µg/kg	CB-85 µg/kg	CB-110 µg/kg	CB-151 µg/kg
2007/0895	A07/041 TX40	500	<23	89	250	61	67	340
2007/0896	A07/014 TX58	530	<24	76	350	66	44	280
2007/0898	A07/033 TX5	440	<58	100	320	36	23	440
2007/0900	A07/034 TX14	350	<27	53	250	39	27	220
2007/0902	A07/016 TX61	1300	55	240	1800	180	190	1000
2007/0904	A07/027 TX10	900	<27	160	590	100	83	570
2007/0906	A07/031 TX26	470	<55	96	690	58	31	440
2007/0908	A07/005 TX13	3700	300	700	2200	580	480	1200
2007/0910	A07/026 TX41	320	<54	88	220	43	38	310
2007/0912	A07/040 TX46	210	<16	37	74	19	39	130
2007/0914	A07/008 TX33	790	<29	120	420	64	60	450
2007/0916	A07/039 TX57	950	<14	110	750	110	110	410

Table 3.2. continuation

LIMS No.	Sample code	CB-149 µg/kg	CB-118 µg/kg	CB-153 µg/kg	CB-141 µg/kg	CB-105 µg/kg	CB-137 µg/kg	CB-138 µg/kg
2007/0895	A07/041 TX40	1000	350	2000	24	100	92	1400
2007/0896	A07/014 TX58	960	460	1700	23	140	15	1300
2007/0898	A07/033 TX5	1600	300	3200	<31	130	2,8	2300
2007/0900	A07/034 TX14	730	330	1400	16	110	5,7	1000
2007/0902	A07/016 TX61	3500	1100	4000	77	300	100	6100
2007/0904	A07/027 TX10	1900	900	3900	42	240	11	2900
2007/0906	A07/031 TX26	1500	390	2800	<29	120	27	2300
2007/0908	A07/005 TX13	3600	3700	1400	190	810	250	1000
2007/0910	A07/026 TX41	850	370	2200	<29	90	<20	1700
2007/0912	A07/040 TX46	360	200	880	11	45	0,8	600
2007/0914	A07/008 TX33	1400	570	2900	43	170	22	2200
2007/0916	A07/039 TX57	1300	770	2400	59	250	25	2000

Table 3.2. Continuation

LIMS No.	Sample code	CB-187 µg/kg	CB-202 µg/kg	CB-128 µg/kg	CB-156 µg/kg	CB-180 µg/kg	CB-170 µg/kg	CB-194 µg/kg	CB-206 µg/kg
2007/0895	A07/041 TX40	770	37	190	<14	260	110	15	<12
2007/0896	A07/014 TX58	520	20	180	4,1	260	97	12	<11
2007/0898	A07/033 TX5	920	18	170	<30	360	130	<23	<25
2007/0900	A07/034 TX14	510	18	130	<14	240	93	12	<12
2007/0902	A07/016 TX61	1600	65	610	2,7	1000	410	43	<24
2007/0904	A07/027 TX10	1500	78	390	<14	1000	200	28	<12
2007/0906	A07/031 TX26	850	26	240	<28	420	160	15	<24
2007/0908	A07/005 TX13	2100	300	1600	87	1100	1500	230	22
2007/0910	A07/026 TX41	730	34	200	<28	180	85	<21	<24
2007/0912	A07/040 TX46	350	19	77	1,8	130	58	10	<7,1
2007/0914	A07/008 TX33	880	18	290	<15	500	200	19	<13
2007/0916	A07/039 TX57	770	31	300	20	400	150	22	<6,0

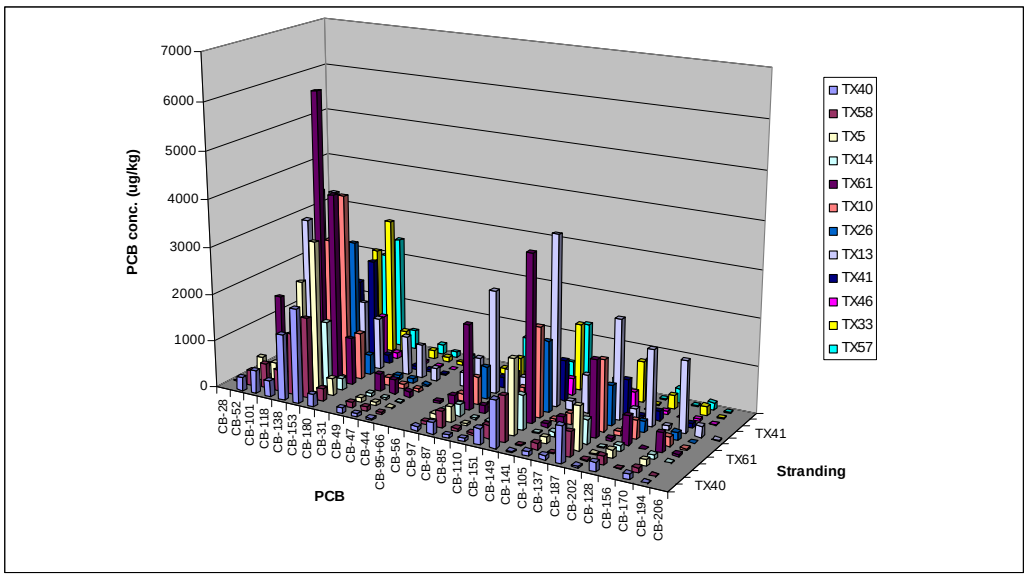

Fig. 3.1. Comparison of distribution of PCB congeners among samples. Values below LOD are not visualized.

Brominated flame retardants

Concentration of brominated flame retardants measured in the blubber samples are given in Table 3.3. and graphical visualization is shown in Fig. 3.2. It should be pointed out that determination of nona brominated flame retardants was performed for the first time. The concentrations of nona BDEs can be an important parameter to asses contamination by deca-BDE, which is still produced and which is known to decompose to lower brominated congeners.

Table 3.3. Concentrations of brominated flame retardants (µg/kg fresh weight) measured in 12 blubber samples.

LIMS No.	Sample code	BDE28 µg/kg	BDE47 µg/kg	BDE49 µg/kg	BDE66 µg/kg	BDE71 µg/kg	BDE75 µg/kg	BDE77 µg/kg
2007/0895	A07/041 TX40	I	140	12	0,9	I	<14	<0,2
2007/0896	A07/014 TX58	I	690	23	4,4	I	<21	1,0
2007/0898	A07/033 TX5	I	110	6,0	0,6	I	<12	<0,06
2007/0900	A07/034 TX14	I	200	13	2,0	I	<8,0	0,4
2007/0902	A07/016 TX61	I	1300	46	5,8	I	44	1,0
2007/0904	A07/027 TX10	I	150	9,0	1,4	I	<8,4	<0,05
2007/0906	A07/031 TX26	I	850	12	2,5	I	<19	0,8
2007/0908	A07/005 TX13	I	490	12	2,6	I	64	1,0
2007/0910	A07/026 TX41	I	120	5,4	0,4	I	<12	0,3
2007/0912	A07/040 TX46	I	51	5,6	0,6	I	<5,6	<0,05
2007/0914	A07/008 TX33	I	160	13	1,2	I	<13	<0,06
2007/0916	A07/039 TX57	I	430	33	6,8	I	<8,1	0,7

I – due to interferences present in the chromatogram, concentration could not be reported

Table 3.3. Continuation

LIMS No.	Sample code	BDE85 µg/kg	BDE99 µg/kg	BDE100 µg/kg	BDE119 µg/kg	BDE138 µg/kg	BDE153 µg/kg	BDE154 + BB153 µg/kg
2007/0895	A07/041 TX40	<0,07	33	42	2,2	2,1	I	15
2007/0896	A07/014 TX58	<0,06	130	220	6,6	3,3	I	39
2007/0898	A07/033 TX5	<0,07	25	34	<1,3	0,5	I	12
2007/0900	A07/034 TX14	<0,06	32	61	2,3	1,5	I	19
2007/0902	A07/016 TX61	<0,07	190	370	7,4	3,3	I	140
2007/0904	A07/027 TX10	<0,07	28	41	2,6	2,1	I	19
2007/0906	A07/031 TX26	<0,07	94	260	5,5	5,2	I	110
2007/0908	A07/005 TX13	<0,07	170	310	8,6	19	I	130
2007/0910	A07/026 TX41	<0,07	12	29	<1,3	1,2	I	15
2007/0912	A07/040 TX46	<0,07	7,8	15	<1,0	1,1	I	7,4
2007/0914	A07/008 TX33	<0,07	18	45	1,9	2,3	I	17
2007/0916	A07/039 TX57	<0,07	72	130	4,6	3,1	I	29

I – due to interferences present in the chromatogram, concentration could not be reported

Table 3.3. Continuation

LIMS No.	Sample code	BDE183 µg/kg	BDE190 µg/kg	BDE209 µg/kg	BDE206 µg/kg	BDE207 µg/kg	BDE208 µg/kg
2007/0895	A07/041 TX40	0,4	<0,07	<2,0	<2,1	<2,3	<2,3
2007/0896	A07/014 TX58	0,5	<0,06	<0,5	<1,8	<2,0	<2,0
2007/0898	A07/033 TX5	0,2	<0,07	<0,6	<2,1	<2,3	<2,3
2007/0900	A07/034 TX14	0,2	<0,06	<0,5	<1,9	<2,1	<2,1
2007/0902	A07/016 TX61	0,7	<0,07	<0,5	<2,0	<2,2	<2,2
2007/0904	A07/027 TX10	0,2	<0,07	<0,6	<2,0	<2,2	<2,2
2007/0906	A07/031 TX26	0,6	<0,08	<0,6	<2,2	<2,5	<2,5
2007/0908	A07/005 TX13	0,9	<0,07	<0,6	<2,2	<2,4	<2,4
2007/0910	A07/026 TX41	0,3	<0,07	<0,6	<2,1	<2,3	<2,3
2007/0912	A07/040 TX46	0,2	<0,07	<0,6	<2,0	<2,2	<2,2
2007/0914	A07/008 TX33	<0,08	<0,07	<0,6	<2,1	<2,3	<2,3
2007/0916	A07/039 TX57	0,3	<0,07	<0,6	<2,1	<2,3	<2,3

Table 3.3. Continuation

LIMS No.	Sample code	Me- TBBP-A µg/kg	TBBP-A µg/kg	α-HBCD µg/kg	β-HBCD µg/kg	γ-HBCD µg/kg
2007/0895	A07/041 TX40	<0,2	<8,4	110	<8,6	<8,5
2007/0896	A07/014 TX58	<0,2	<7,4	720	<7,5	<7,5
2007/0898	A07/033 TX5	<0,2	<8,5	130	<8,6	<8,5
2007/0900	A07/034 TX14	<0,2	<7,8	110	<7,9	<7,9
2007/0902	A07/016 TX61	<0,2	<8,0	920	<8,1	<8,0
2007/0904	A07/027 TX10	<0,2	<8,1	110	<8,2	<8,1
2007/0906	A07/031 TX26	<0,2	<9,1	590	<9,2	<9,1
2007/0908	A07/005 TX13	<0,2	<9,0	1400	<9,1	<9,0
2007/0910	A07/026 TX41	<0,2	<8,6	38	<8,7	<8,6
2007/0912	A07/040 TX46	<0,2	<8,2	19	<8,3	<8,2
2007/0914	A07/008 TX33	<0,2	<8,5	170	<8,6	<8,5
2007/0916	A07/039 TX57	<0,2	<8,5	220	<8,6	<8,5

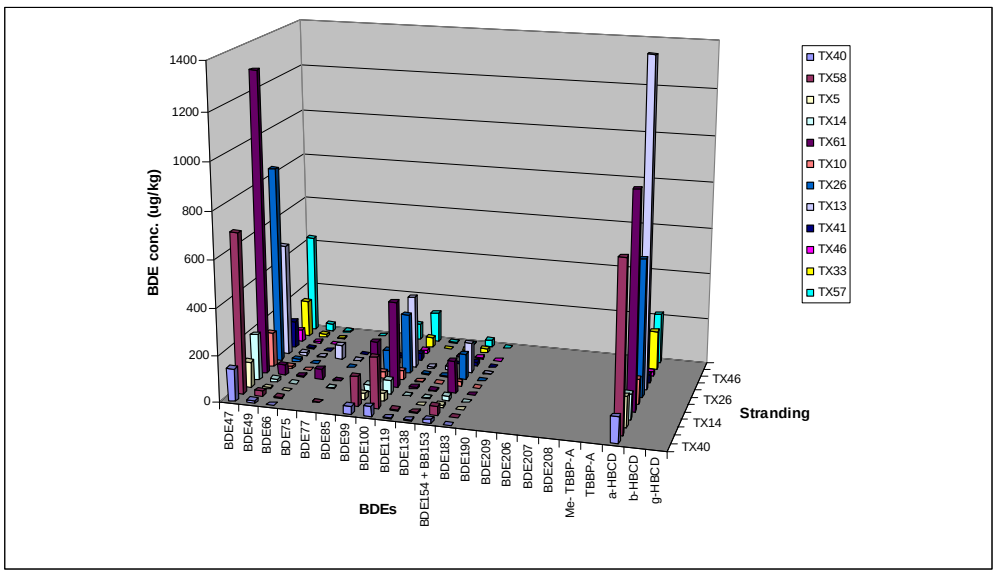

Fig. 3.2. Comparison of distribution of flame retardants among samples. Values below LOD are not visualized.

Perfluorinated compounds

Concentrations of perfluorinated compounds measured in liver samples are given in Table 3.4. and graphical visualization is shown in Fig. 3.3. Although analyses of only 2 PFCs were contracted (PFOS and PFOA), 9 additional perfluorinated compounds were performed to provide more comprehensive characterization of PFCs distribution in the harbour porpoise samples. Comparison of the PCB patterns among the harbour porpoise samples can provide information on uniformity of the contamination source and comparison between PCB pattern in harbour porpoises and their preys can be a useful tool to trace the uptake route.

Table 3.4. Concentrations of perfluorinated compounds (µg/kg fresh weight) measured in 12 liver samples.

LIMS No.	Sample code	PFOA µg/kg	PFOS µg/kg	PFHxA µg/kg	PFNA µg/kg	PFUnA µg/kg	PFBA µg/kg
2007/0894	A07/041 TX40	<2,4	290	<2,2	1,5	9,5	<2,3
2007/0897	A07/014 TX58	<2,9	610	<2,7	8,4	23	<2,8
2007/0899	A07/033 TX5	<3,9	1300	<3,6	5,7	40	<3,8
2007/0901	A07/034 TX14	<3,6	1100	<3,3	4,8	39	<3,5
2007/0903	A07/016 TX61	<3,8	740	<3,5	5,8	130	<3,7
2007/0905	A07/027 TX10	<3,7	270	<3,5	1,1	15	<3,7
2007/0907	A07/031 TX26	<2,9	300	<2,7	4,3	8,2	<2,9
2007/0909	A07/005 TX13	<3,8	1700	<3,5	14	42	<3,7
2007/0911	A07/026 TX41	<3,3	1300	<3,0	3,1	30	<3,2
2007/0913	A07/040 TX46	<3,7	1300	<3,5	5,1	49	<3,7
2007/0915	A07/008 TX33	<3,9	3000	<3,6	11	48	<3,8
2007/0917	A07/039 TX57	<3,7	1700	<3,4	10	77	<3,6

Table 3.4. Continuation

LIMS No.	Sample code	PFBS µg/kg	PFDoA µg/kg	PFDcA µg/kg	PFOSA µg/kg	PFHxS µg/kg
2007/0894	A07/041 TX40	<2,0	<4,2	12	12	<4,1
2007/0897	A07/014 TX58	<2,4	<5,1	18	<2,8	5,7
2007/0899	A07/033 TX5	<3,3	<6,9	49	22	8,1
2007/0901	A07/034 TX14	<3,0	<6,3	35	35	4,0
2007/0903	A07/016 TX61	<3,2	31	45	<3,6	2,8
2007/0905	A07/027 TX10	<3,2	<6,6	8,5	10	<6,4
2007/0907	A07/031 TX26	<2,5	<5,2	15	7,3	<5,1
2007/0909	A07/005 TX13	<3,2	<6,6	65	28	8,2
2007/0911	A07/026 TX41	<2,8	<5,8	40	<3,1	7,8
2007/0913	A07/040 TX46	<3,2	<6,6	54	30	<6,5
2007/0915	A07/008 TX33	<3,3	<6,9	49	<3,7	11
2007/0917	A07/039 TX57	<3,1	<6,5	65	24	4,5

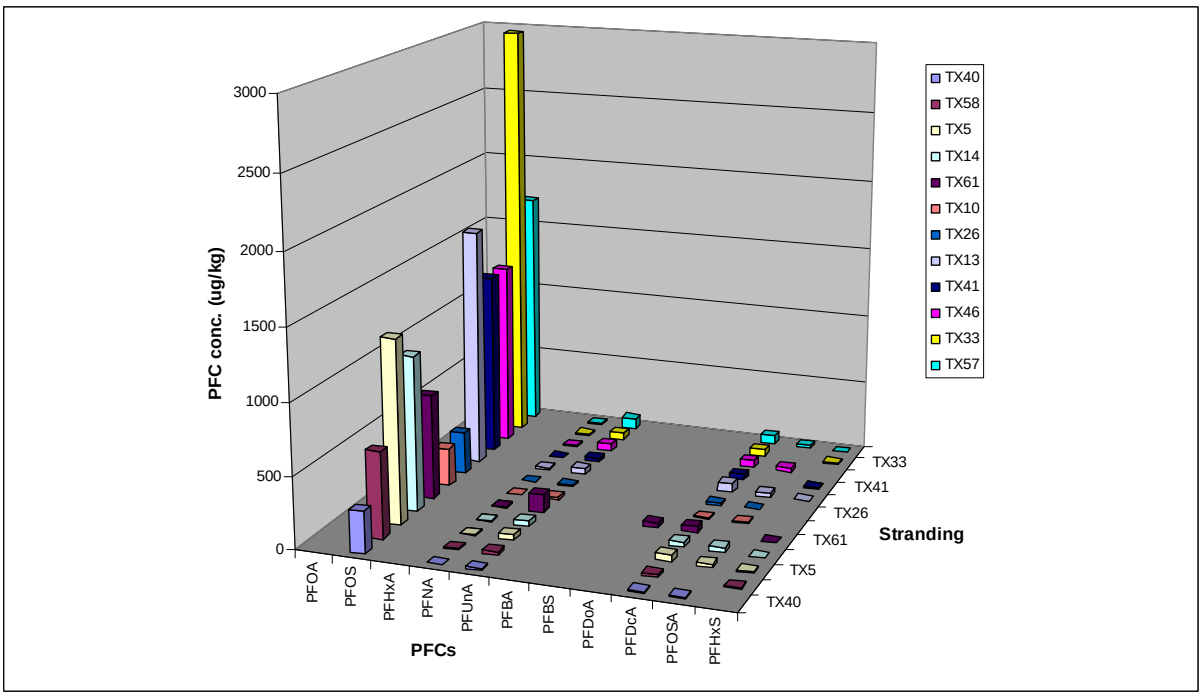

Fig. 3.3. Comparison of distribution of perfluorinated compounds among samples. Values below LOD are not visualized.

Organotin compounds

Concentrations of organotin compounds measured in liver samples are given in Table 3.5. and graphical visualization is shown in Fig. 3.4. The method for analysis of organotin compounds was specifically implemented for this project. Exactly the same method as used by RIKZ, Haren was implemented to ensure comparability and consistency of the results, which are important factors when trend analysis is required. Help of RIKZ, Haren, especially Ton van der Zande, is highly appreciated.

Table 3.5. Concentrations of organotin compounds (µg Sn/kg fresh weight) measured in 12 liver samples.

LIMS No.	Sample code	Tributyltin µg Sn/kg	Dibutyltin µg Sn/kg	Monobutyltin µg Sn/kg	Triphenyltin µg Sn/kg	Diphenyltin µg Sn/kg	Monophenyltin µg Sn/kg
2007/0894	A07/041 TX40	25	25	<2,6	2,8	<2,7	<2,7
2007/0897	A07/014 TX58	13	26	<2,1	<2,0	<2,2	<2,2
2007/0899	A07/033 TX5	26	35	<1,1	<1,0	<1,1	<1,1
2007/0901	A07/034 TX14	7,9	29	<2,3	<2,2	<2,4	<2,4
2007/0903	A07/016 TX61	60	240	7,3	5,4	<3,0	<3,0
2007/0905	A07/027 TX10	9,3	11	<2,0	<1,9	<2,1	<2,1
2007/0907	A07/031 TX26	27	140	<1,7	<1,6	<1,8	<1,8
2007/0909	A07/005 TX13	68	170	<1,3	2,8	<1,4	<1,4
2007/0911	A07/026 TX41	22	23	<1,8	2,8	<1,8	<1,9
2007/0913	A07/040 TX46	71	140	2,1	4	<1,9	<2,0
2007/0915	A07/008 TX33	23	250	14	<2,2	<2,4	<2,4
2007/0917	A07/039 TX57	41	44	<3,3	<3,1	<3,5	<3,5

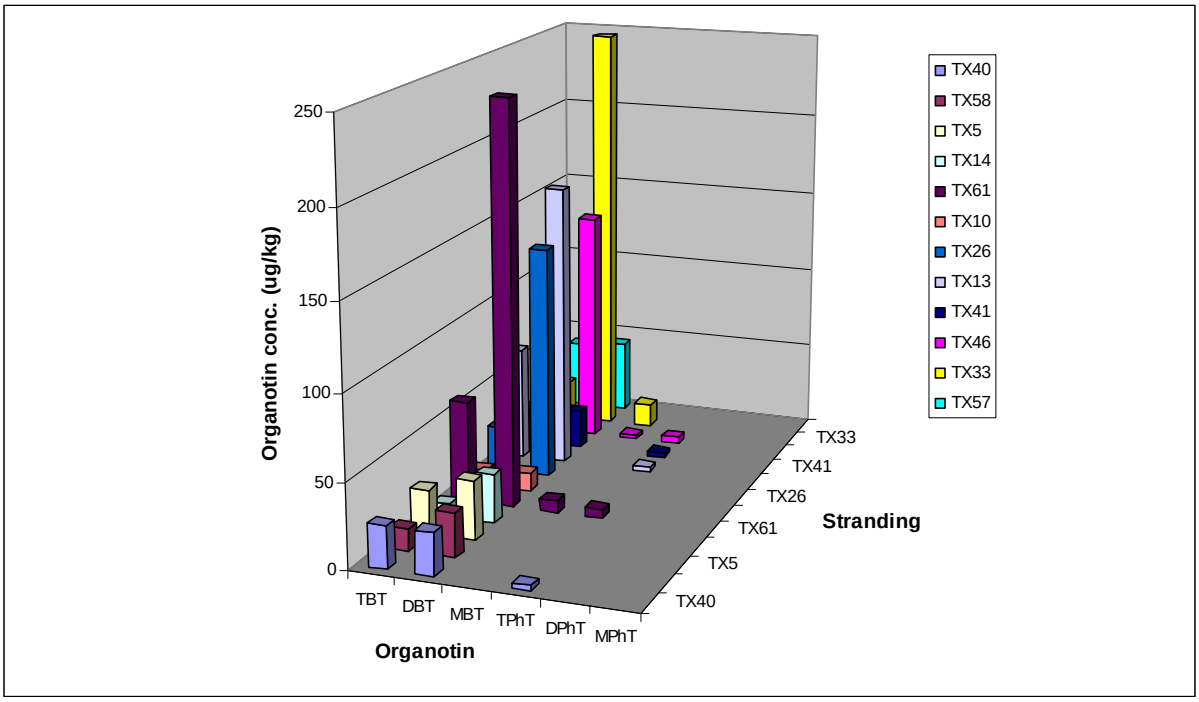

Fig. 3.4. Comparison of distribution of organotin compounds among samples. Values below LOD are not visualized.

4. Quality characteristics

IMARES utilises an ISO 9001:2000 certified quality management system (certificate number: 08602-2004-AQ-ROT-RvA). This certificate is valid until 15 December 2009. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. The last certification inspection was held the 16-22 of May 2007. The chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2009 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation, with the last inspection being held on the 5th of October 2007.

Determination of dry weight, fat content, protein content, PCBs and flame retardants were performed using methods ISO 17025:2005 accredited for measurements in fish and fishery products. Analyses of harbour porpoise, as analysis of mammals, does not comply with the scope of accreditation, but based on our experience the harbour porpoise matrix is very similar with fish and fishery product matrices. Determination of perfluorinated and organotin compounds was performed by validated methods. In each determination, high quality control measures required by ISO 17025:2005 accreditation were applied. Internal reference material (IRM) and/or certified reference material (CRM), blanco, recovery standard and one sample in duplicate were analysed in each serie of samples. In addition, quality of IMARES laboratory is ensured by regular participation in the interlaboratory studies for all determinants except the organotin compounds. Comparability test of IMARES organotin method with RIKZ method was performed by analysis of internal reference material provided by RIKZ, Haren and in the future participation in the interlaboratory testing scheme for organotin is planned.

One should remember that each analytical determination is always accompanied by some uncertainty. When trend analysis or differences among samples are evaluated, the uncertainty should be incorporated into the statistical evaluation. IMARES follows the highest quality control measures and has a deep insight into estimation of the uncertainties. In case of interest, IMARES is prepared to advice on the uncertainties and the statistical evaluation of the data.

References

Leopold, M.F. & Camphuysen, 2006. Bruinvisstrandingen in Nederland in 2006. Achtergronden, leeftijdsverdeling, sexratio, voedselkeuze en mogelijke oorzaken. C083/06. Texel, Wageningen IMARES.

Referees and Authors

Rapport C140/07
Project Number: 4395101101

This report has been professionally prepared by Wageningen IMARES. The scientific validity of this report has been internally tested and verified by another researcher and evaluated by the Scientific Team at Wageningen IMARES.

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Head department Environment

Signature:

Date: December 15th 2007

Number of copies:	9
Number of pages	19
Number of tables:	6
Number of graphs:	8