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A first overview of contaminants in all age classes

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Contents

Summary

This report presents a first overview of contaminant levels in all age classes of both male and female harbour porpoises beached along the Dutch coast. Both neonates (new-born calves) and juveniles obtain high levels of different types of contaminant groups during their early life stage, up to levels that may exert physiological or reproductive effects. Adult male porpoises accumulate the highest PCB and HCB levels in their blubber relative to other age classes and the female sex. Adult female porpoises can offload their PCB and HCB burden via placenta transfer and especially lactation on to their calves. One adult female showed signs of reproduction failure in combination with deviating (high) PCB and PBDE concentrations.

This study as well as international studies show that both reproduction success and the immune system of harbour porpoises can be affected by contaminants, already in their early life stages. This may have consequences for the stability of the porpoise population in the southern North Sea. Further insight into the role of contaminants on the fitness of harbour porpoises is therefore urgently needed.

1 Introduction

Rijkswaterstaat established the 'Wind op Zee Ecologisch Programma' (Wozep) commissioned by the Ministry of Economic Affairs, in order to investigate knowledge gaps concerning the effects of wind farms on the ecology in the North Sea (Rijkswaterstaat 2016). One of the key species within this program is the harbour porpoise, *Phocoena phocoena*. As being one of the top predators (Peltier et al. 2013), these animals may reflect environmental changes, also as an effect of anthropogenic activities in their habitat. Within the framework of Wozep, a set of 'no-regret' studies was requested in 2016 by Rijkswaterstaat. This included research on direct impact of windfarm construction and cumulative threats on the harbour porpoise population. One of the possible cumulative threats is contamination, as presented in this report. Related results on acoustic trauma and life history (age composition and reproduction) of subsets of harbour porpoises stranded on the Dutch coast is presented elsewhere (IJsseldijk & Gröne, 2016).

Contaminants form a potential harm to the harbour porpoise, because they can negatively affect the immune system (increased risk for infections), the hormone system and reproduction (Camphuysen & Siemensma, 2011). These contaminants are a.o. chlorinated compounds (such as PolyChlorinatedBiphenyls – PCBs), brominated compounds (such as PolyBrominatedDiphenylEthers – PBDEs), organotin compounds (such as TriButylTin – TBT) and perfluorinated compounds (PFCs)(Table 1). British studies relate PCB contamination in harbour porpoises to reproduction problems within their population, which points at possible risks for population growth and stability of the harbour porpoise population in the North Sea (Murphy et al. 2015; Jepson et al. 2016). These compounds are taken up by harbour porpoises via their food and are known to be bioaccumulative. This means that concentrations increase when the compounds are being transferred up the food web (Van den Heuvel-Greve & Zabel, 2010). An international study (BIOCET) on effects of pollutants in marine mammals in the North Sea showed that a substantial proportion of individuals in their study had contaminant levels above the PCB threshold level for adverse effects, especially in individuals from the southern North Sea (Murphy et al., 2010).

In previous years (2009-2015) chemical analyses in the Netherlands have been mainly conducted in samples of the juvenile age class of beached harbour porpoises, focusing on PCBs, PBDEs and in some cases also TBT and PFCs (Van den Heuvel-Greve et al., 2011). Since 2014, PCBs and PBDEs have also been analysed in the neonate age class and some adult females (Van den Heuvel-Greve et al., 2014; Van den Heuvel-Greve et al., 2016). A full overview of contaminant pressure in all age classes of both male and female harbour porpoises beached along the Dutch coast line has not been made before.

This report contains the results on the analysis of contaminants in beached adult harbour porpoises, directly combined with data from previous years to obtain a first overview of contaminant concentrations in all age groups of harbour porpoises beached along the Dutch coast.

Table 1

Effects on the immune and reproduction system of marine mammals as published in scientific literature (based on Van den Heuvel-Greve & Zabel, 2010). ↓ indicates a decreased functioning, ↑ indicates an increased functioning, ↓↑ indicates alterations.

Contaminant	Abbreviation	Effect on immune system	Effect on reproduction system	Main use
PolyChlorinated Biphenyls	PCBs	↓ (in seals, fur seals and sea otters)	↓ (in seals)	Additive in chemical industry since 1929 (e.g. fluid in electrical systems, hydraulic lubricants, sealants)
PolyBrominated DiphenylEthers	PBDEs	↓↑ (in seals)	↓ (in seals)	Additive in fire retardants: since 1960s in polymers and textiles and 1970s in electronic equipment
Organotin Compounds TriButylTin	OTCs TBT	↓ (in sea otters)	- (↓ in marine snails)	Additive since 1960s as biocide, fungicide and stabilizer (e.g. antifouling paints, wood preservation, polymers)
Perfluorinated Compounds	PFCs	- (↓ in mice)	↓ (in dolphins)	Additive used since 1950s. Wide range of applications (e.g. surfactant, refrigerant, fire retardant, pharmaceuticals)

2 Assignment

The research question of this report is: can contaminants form a serious pressure on the stability of the harbour porpoise population in the southern North Sea?

This research question will be addressed focusing on the following sub questions:

1. What are contaminant levels in adult harbour porpoises beached along the Dutch shorelines?
2. Do age and length influence contaminant concentrations in harbour porpoises beached along the Dutch shorelines?
3. Does number of ovulations influence contaminant concentrations in harbour porpoises beached along the Dutch shorelines?
4. Do contaminants form a potential pressure on the harbour porpoise population in the North Sea?

3 Materials and Methods

Since 2008, a structured post mortem investigation on stranded harbour porpoises in The Netherlands has been conducted at the faculty of Veterinary Medicine at Utrecht University, commissioned by the Ministry of Economic Affairs. During these investigations the following samples are stored for contaminant studies: blubber, liver, muscle and kidney. The samples are taken and stored according to standard protocols for contaminant research, as developed by Wageningen Marine Research. Sample storage is at -20°C. Wageningen Marine Research conducts contaminant research on these samples.

Table 2 shows the details of harbour porpoise samples (n=17) that were analysed in this study and were added to the existing database. The added samples consisted of adult males and females that stranded along the Dutch coastline between 2014-2016. Samples were selected based on their decomposition condition code (DCC = 1-6, with 1 being fresh samples and 6 being extremely decomposed samples; only fresh to moderately fresh samples with an DCC code of 3 or less were selected) and nutritional condition code (NCC = 1-6, with 1 being well fed individuals and 6 being extremely emaciated individuals; samples of harbour porpoises with a moderate to good nutritional status of NCC code of 4 or less were preferred, but due to a low availability of samples sometimes also NCC codes of >4 had to be included). This selection reduces variation in results as decomposition may degrade contaminant levels and emaciation may concentrate contaminant levels in blubber.

In previous years samples have been analysed for PCBs, PBDEs and PFCs in several age groups. The presented results in this report show the combined datasets of this year and the previous years (individuals beached between 2006 – 2016). The total number of analysed samples differs per contaminant groups as methods differ for chemical analysis and different sets of samples have been analysed for different contaminant groups in the past years. Most samples have been analysed for PCBs, followed by PBDEs and then PFCs. The number of samples analysed per contaminant are presented in Annex H.

Age groups were determined by the University of Utrecht during necropsy based on visual characteristics. Individuals were characterised as neonate based on time of stranding during the birth period (May-June-July) in combination with length. The following age groups of harbour porpoises are used in this report:

- Foetus: unborn calve (in uterus). The foetus samples in the dataset have a body length of 28-71 cm.
- Neonate: new-born calve, still consuming milk. The neonates samples in the dataset have a body length of 69-84 cm. Neonates are on average 65-75 cm at time of birth (Lockyer, 2003; Lockyer & Kinze, 2003).
- Juvenile: non-milk drinking, non-reproducing porpoises. The juvenile samples in the dataset have a body length of 87-122 cm.
- Adult: reproducing porpoises. The adult samples in the total dataset have a body length of 128-166 cm.

Table 2

Characteristics of beached adult harbour porpoises, that were used for contaminant analyses in this report. Date is date beached individual was found. DCC = decomposition condition code of the sample (the higher the number the more the sample is decomposed on a scale of 1-6). NCC = nutritional condition code of the beached harbour porpoise (the higher the number the worse the nutritional status is on a scale of 1-6).

										cm	kg		
Species	**Carcass**	**Sample**	**Year of analysis**	**Dd**	**Mm**	**Yy**	**Age class**	**Age**	**Sex**	**Total length**	**Mass**	**DCC**	**NCC**
Harbour porpoise	UT1311	Blubber/liver	2016	3	3	2014	A	5	F	158	54	2	2
Harbour porpoise	UT1316	Blubber/liver	2016	25	6	2014	A	6	F	153	48	1	5
Harbour porpoise	UT1448	Blubber/liver	2016	4	3	2015	A	4	F	153	48	2	2
Harbour porpoise	UT1467	Blubber/liver	2016	31	5	2015	A	10	M	141	39	2	6
Harbour porpoise	UT1470	Blubber/liver	2016	24	6	2015	A	7	F	153	38	2	5
Harbour porpoise	UT1472	Blubber/liver	2016	3	7	2015	A	5	M	138	34	2	5
Harbour porpoise	UT1482	Blubber/liver	2016	1	8	2015	A	7	F	155	34	2	6
Harbour porpoise	UT1484	Blubber/liver	2016	18	8	2015	A	8	F	164	40	1	5
Harbour porpoise	UT1492	Blubber/liver	2016	3	1	2016	A	15	M	137	41	1	1
Harbour porpoise	UT1494	Blubber/liver	2016	2	2	2016	A	5	M	128	30	3	3
Harbour porpoise	UT1496	Blubber/liver	2016	29	2	2016	A	3	F	150	45	3	1
Harbour porpoise	UT1508	Blubber/liver	2016	10	3	2016	A	11	F	158	58	2	1
Harbour porpoise	UT1512	Blubber/liver	2016	22	3	2016	A	6	M	130	31	2	4
Harbour porpoise	UT1522	Blubber/liver	2016	18	5	2016	A	6	M	143	48	2	2
Harbour porpoise	UT1527	Blubber/liver	2016	29	6	2016	A	8	F	157	39	1	6
Harbour porpoise	UT1531	Blubber/liver	2016	7	7	2016	A	5	M	139	42	1	3
Harbour porpoise	UT1535	Blubber/liver	2016	28	7	2016	A	6	F	146	46	1	6

3.1 Chemical analysis

PCBs (including dioxin-like PCBs), PBDEs, HCB, HCBD were analysed in blubber samples, whereas PFCs and mercury (Hg) were analysed in liver samples. These tissues were selected based on the preference of the respective contaminants for internal storage in mammals.

3.1.1 Homogenisation

In order to obtain proper analytical samples of liver and blubber, samples were homogenised prior to analysis. The liver samples were homogenised with a blender. Blubber samples required more preparation. First the outside of the blubber (typically a piece of the blubber including the skin) was cut “clean” and then a non-air exposed part of the blubber was excised, without the skin. As the blubber was too flexible and tough to be homogenised by a blender, it was cut into very small parts. The samples were then used for analyses on the Wageningen Marine Research laboratory in IJmuiden.

3.1.2 Lipid content and dry weight

The lipid levels were determined by a method, modified from the Bligh & Dyer (B&D) method (De Boer, 1988). This method is ISO 17025:2005 accredited, SOP 2.10.3.002 “Animal tissue. Determination of the fat content was conducted according to Bligh and Dyer” (test laboratory L097, determination number 1). Samples were extracted three times with a mix of chloroform, methanol and demineralised water. Lipid level was determined by weighing the residue after evaporation of the solvent.

Dry weight was determined according to ISO 17025:2005 accredited SOP 2.10.3.011 “Animal tissue. Determination of the level of moisture”. Samples of approximately 1 gram were dried for 3 hours at 105 °C after which the dry weight was determined gravimetrically.

3.1.3 Analysis of PCBs, PBDEs, HCBD and HCB

The Accelerated Solvent Extraction (ASE) and gas chromatography coupled to a mass spectrometry (GC-MS) method was applied for PCBs, PBDEs, HCB and HCBD. This method (SOP 2.10.3.050 Biota and environmental matrices: Determination of micro pollutants after ASE extraction and GCMS detection) is validated for biota according to ISO 17025. The sample was mixed with sodium sulphate and transferred to an ASE cell containing 25 g Florisil. The ASE cell was extracted 3 times using

pentane/dichloromethane (85:15). After addition of 1 ml of iso-octane as a keeper, the extract was concentrated to 1 ml in a rotary vapour. PCBs, PBDEs, HCB and HCBD can then be determined by a GC-MS detector. Calibration was performed using certified standards and at least a six point calibration curve. Along with each set of samples a blank and an internal reference sample was extracted and measured.

All PCBs that could be quantified (maximum of 28 congeners in the calibration standards) were quantified in the samples. In the samples 20-24 congeners were quantified. For PBDEs 15 congeners could be quantified with the calibration standards, up to 12 congeners were detected in the samples. See Annex F for all analysed congeners.

Twelve of the PCB congeners have dioxin-like activities. These are the coplanar PCBs that consist of both the non-ortho and mono-ortho PCBs. Non-ortho PCBs analysed in this study were: PCB77, PCB81, PCB126, PCB169. Mono-ortho PCBs analysed in this study were: PCB105, PCB114, PCB118, PCB123, PCB 156, PCB157, PCB167, PCB189. See Annex F.

For comparing concentrations of PCBs, PBDEs, HBCD and HCB in harbour porpoises, concentrations were based on 100% lipid.

3.1.4 Analysis of PFCs

Perfluorinated compounds were determined in liver by accredited method (SOP 2.10.3.045 Determination of the content of perfluoroalkylated substances after extraction). Samples were extracted 3 times using 10 ml acetonitrile. Extracts were dried using sodium sulphate, concentrated to 1 ml by turbovap and transferred to a centrifuge tube containing 50 mg ENVIcarb. Tubes were vortexed for 30 seconds and subsequently centrifuged. Extracts were transferred to a vial and analyzed by liquid chromatography coupled with a mass spectrometry detector (LC-MS). Calibration was performed using at least a six point calibration curve. Along with each set of samples a blank and an internal reference sample were extracted and measured. No PFCs were detected in the blanks.

For comparing concentrations of PFCs in harbour porpoises, concentrations were based on 100% dry matter. As PFCs do not accumulate in lipid, standardisation on lipid weight is not of use and will add an error to the data. To reduce variation between samples, concentrations were therefore based on dry weight as wet content may differ between samples.

3.1.5 Analysis of Mercury

For the determination of the levels of mercury, samples were dried and then destructed (at high temperature). With supply of oxygen the volatiles were led to a catalyst tube, where oxidation takes place and halogens, nitrogen and sulphur oxides are removed. The residual destruction products were led to an amalgamator, what converts mercury compounds into metallic mercury. The level of mercury was than quantified by use of a flame-less atomic absorption spectrometer. The samples were measured against a calibration curve, which consists of a certified reference material, analysed in the same manner at different quantities. The method is registered in WMR SOP 2.10.3.025 “Dierlijk weefsel. Bepalen van het gehalte aan kwik m.b.v. SMS100 mercury analyser; vlamloze AAS” and is accredited by the council for accreditation (RvA) (testlaboratory number L097, method number 6).

Although this method has shown to be very versatile and robust, the porpoise livers could not be analysed by the apparatus. The responses of mercury were very low, apparently caused by an incomplete combustion and very rapid "soiling" of the whole system. No mercury results could therefore be included in this report.

3.2 Statistical treatment

To obtain an overview of contaminant levels in all age classes of harbour porpoises beached along the Dutch coast, all data on contaminant levels in these harbour porpoises, as analysed over the past ten years, were combined. See Annex H for the number of samples per contaminant, age group and sex.

Sum-parameters of PCBs and PBDEs were calculated. Sum-17PCB consists of CB49, CB52, CB101, CB105, CB118, CB128, CB138, CB149, CB151, CB153, CB156, CB170, CB187, CB180, CB194CB202. Sum-7PCB consists of CB52, CB101, CB118, CB138, CB153, CB180. Sum-6PBDE consists of BDE 28, 47, 99, 100, 153 and 154. These were chosen as the Environmental Quality Standards (EQS) within the Water Framework Directive are based on these congeners (EC, 2013).

For PCBs and PBDEs statistical treatments were possible. For PFCs and HCB number of data were too low for statistical analysis. The differences in PCBs and PBDEs between sex and age were analysed in R by means of a two way ANOVA for unbalanced designs. Before analysis the data were log(X+0.001)-transformed to normalise the data. A significant interaction between age and sex was found for PCBs. This means that per age group female and males showed different patterns. Therefore separate effects of age and sex could not be statistically tested for PCBs. Interactions for PBDE data were not significant. Therefore, both age and sex could be statistically tested for PBDEs.

4 Results and discussion

The results of the data are reported with a decimal point (.) instead of a comma (,) (in derogation of the Dutch SI). The results stated in this report apply only to the samples analysed for this project and samples analysed in previous related projects conducted by Wageningen Marine Research/IMARES. Information on number of samples per contaminant, age group and sex can be found in Annex H.

4.1 Polychlorinated Biphenyls (PCBs)

4.1.1 Levels in harbour porpoises

When looking at all PCB data in samples of harbour porpoise beached along the Dutch coast between 2006-2016 (both data from this study and earlier reported data, see Annex H), Sum-PCB levels in blubber were 0.02 – 56.1 mg/kg ww (wet weight) (Table 3) and 0.2 – 80 mg/kg lw (lipid weight) (Table 4). This corresponds with earlier published levels of PCBs harbour porpoises in the UK (0.4 – 160 mg/kg lw; Jepson et al., 2016) and North Sea (Table 5) (Weijs et al., 2009; Weijs et al., 2020; Mahfouz et al., 2014). Not enough samples were analysed per age group, sex and year to asses time trends. The largest dataset on PCBs in juveniles only have multiple data points for 2006, 2008 and 2009 and only one or two measurements in 2007, 2010 and 2011. Variation between age groups is too high to assess trends in time based on all age groups.

The NCC of the beached porpoises influenced the PCB concentrations in blubber. Higher PCB concentrations were observed in porpoises with a poor nutritional status (NCC>4) than in porpoises with a moderate to good nutritional status (NCC<4)(see Annex A). During starvation harbour porpoises consume their fat reserves, which are mainly present in their blubber layer, for energy production. This may result in an increased concentration of the PCBs in blubber due to the decreasing blubber volume (less blubber, but same amount of PCBs). Additionally, some of the stored PCBs may be released from the blubber and redistributed into the body with the potential for physiological effects (Van den Heuvel-Greve et al., 2011). As the NCC affects PCB concentrations, samples from porpoises with an NCC>4 were in certain cases omitted for a better comparison between groups of porpoises. This is stated in the respective figures.

PCB levels were relatively low in foetuses of harbour porpoises and higher in samples of neonates, juveniles and adults (Figure 1, 2, Table 5 and Annex A). Statistical analysis showed that for PCBs there is a significant interaction between age group and sex (see Annex G). This means that levels in males and females vary between age groups. In neonates, concentrations were higher in females than males. There is currently no explanation for this observation and this may be due to the low number of samples analysed. In adults, concentrations in males were higher than in females. This can be explained by the fact that females offload their PCB concentrations and therefore decrease their body burden through lactation (Murphy et al., 2015).

Harbour porpoises receive low levels of PCBs in their foetal stage through transfer via the placenta (figure 1). All four foetus samples analysed in this study contained relatively low Sum-17PCB concentrations. Lactation is the main route for transfer of PCBs from adult females to their offspring, especially during the first 7-8 weeks of lactation (Murphy et al., 2015). Juvenile concentration may be further increased by consuming contaminated food. Adult males possessed highest PCB concentrations due to PCB intake via all routes combined: placenta, lactation and diet, without the potential of offloading (figures 1 and 2).

Table 3

Levels in samples of harbour porpoises, beached along the Dutch coast, in the period 2006-2016 (based on wet weight).

Compound	n	Sample		Average	Stdev	Median	Range
Sum-17PCB	91	Blubber	mg/kg ww	10.2	11.2	6.9	0.02 – 56.1
Sum-6PBDE	78	Blubber	mg/kg ww	0.30	0.39	0.16	0.002 – 2.000
HCB	18	Blubber	mg/kg ww	0.1	0.2	0.1	0.013 – 0.560
Sum 6-dioxin-like PCB	21	Blubber	mg/kg ww	0.25	0.30	0.16	0.04 – 1.46
Sum 6-dioxin-like PCB	21	Blubber	ng/kg TEQ ww	23	21	23	1 - 86
Sum-PFC	59	Liver	mg/kg ww	0.7	0.6	0.5	0.06 – 3.1
PFOS	59	Liver	mg/kg ww	0.6	0.6	0.4	0.05 – 3.0

Table 4

Levels in samples of harbour porpoises, beached along the Dutch coast, in the period 2006-2016 (based on lipid weight). PFCs/PFOS are not expressed based on lipid weight due to the characteristics of the compounds and therefore not included in this table.

Compound	n	Sample		Average	Stdev	Median	Range
Sum-17PCB	91	Blubber	mg/kg lw	13.1	17.5	8.1	0.2 – 79.5
Sum-6PBDE	78	Blubber	mg/kg lw	0.38	0.57	0.18	0.002 – 2.170
HCB	18	Blubber	mg/kg lw	0.2	0.2	0.1	0.015 – 0.586
Sum 6-dioxin-like PCB	21	Blubber	mg/kg lw	0.34	0.35	0.23	0.05 – 1.64
Sum 6-dioxin-like PCB	21	Blubber	ng/kg TEQ lw	29	24	26	3 - 97

Table 5

PCB levels in samples of different age groups of harbour porpoises in the Dutch North Sea region (present study and Weijset al., 2009 and 2010) and southern North Sea (Mahfouz et al., 2014). Fo = foetus, N = neonate, J = juvenile, A = adult, M = male, F = female.

Compound	n	Age group		Year	Location	Range	Reference of source
Sum-PCBs	12	JM	mg/kg lw	1999-2004	North Sea	12.7 – 33.8	Weijs et al., 2009
	9	JF	mg/kg lw	1999-2004	North Sea	6.5 – 91.5	Weijs et al., 2009
	8	AM	mg/kg lw	1999-2004	North Sea	2.2 – 171.7	Weijs et al., 2009
	5	AF	mg/kg lw	1999-2004	North Sea	3.9 – 21.5	Weijs et al., 2009
Sum-PCBs	1	N	mg/kg lw	1990-1998	North Sea	13.7	Weijs et al., 2010
	4	J	mg/kg lw	1990-1998	North Sea	8.2 – 19.1	Weijs et al., 2010
	1	A	mg/kg lw	1990-1998	North Sea	81.5	Weijs et al., 2010
	2	N	mg/kg lw	2000-2008	North Sea	4.7 - 29	Weijs et al., 2010
	16	J	mg/kg lw	2000-2008	North Sea	1.1 – 68.2	Weijs et al., 2010
	2	A	mg/kg lw	2000-2008	North Sea	15.3 – 34.5	Weijs et al., 2010
Sum-PCBs	12	JM	mg/kg lw	2010-2013	North Sea	0.6 - 110	Mahfouz et al., 2014
	3	JF	mg/kg lw	2010-2013	North Sea	7.4 - 48	Mahfouz et al., 2014
	1	AM	mg/kg lw	2010-2013	North Sea	22	Mahfouz et al., 2014
	4	AF	mg/kg lw	2010-2013	North Sea	2.5 - 7	Mahfouz et al., 2014
Sum-PCBs	2	FoM	mg/kg lw	2006-2016	North Sea	0.61 – 0.63	Present study
	2	FoF	mg/kg lw	2006-2016	North Sea	1.0 – 4.1	Present study
	9	NM	mg/kg lw	2006-2016	North Sea	1.9 – 16.7	Present study
	5	NF	mg/kg lw	2006-2016	North Sea	3.4 – 24.2	Present study
	30	JM	mg/kg lw	2006-2016	North Sea	2.6 – 76.1	Present study
	8	JF	mg/kg lw	2006-2016	North Sea	4.7 – 79.5	Present study
	7	AM	mg/kg lw	2006-2016	North Sea	3.9 – 62.8	Present study
	26	AF	mg/kg lw	2006-2016	North Sea	0.2 – 77.2	Present study

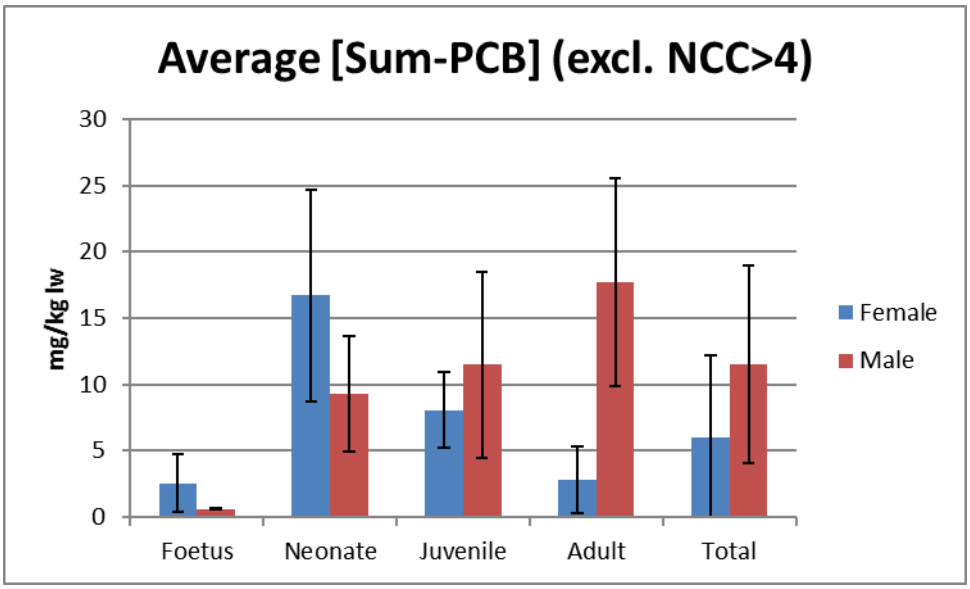

Figure 1 *Sum-17PCB concentrations (in mg/kg lw) in a selection of blubber samples of different age groups of harbour porpoises, beached along the Dutch coast (based on lipid weight). Results of samples of harbour porpoises with a NCC>4 (poor nutritional status) were excluded. See Annex H for number of samples per age group and sex.*

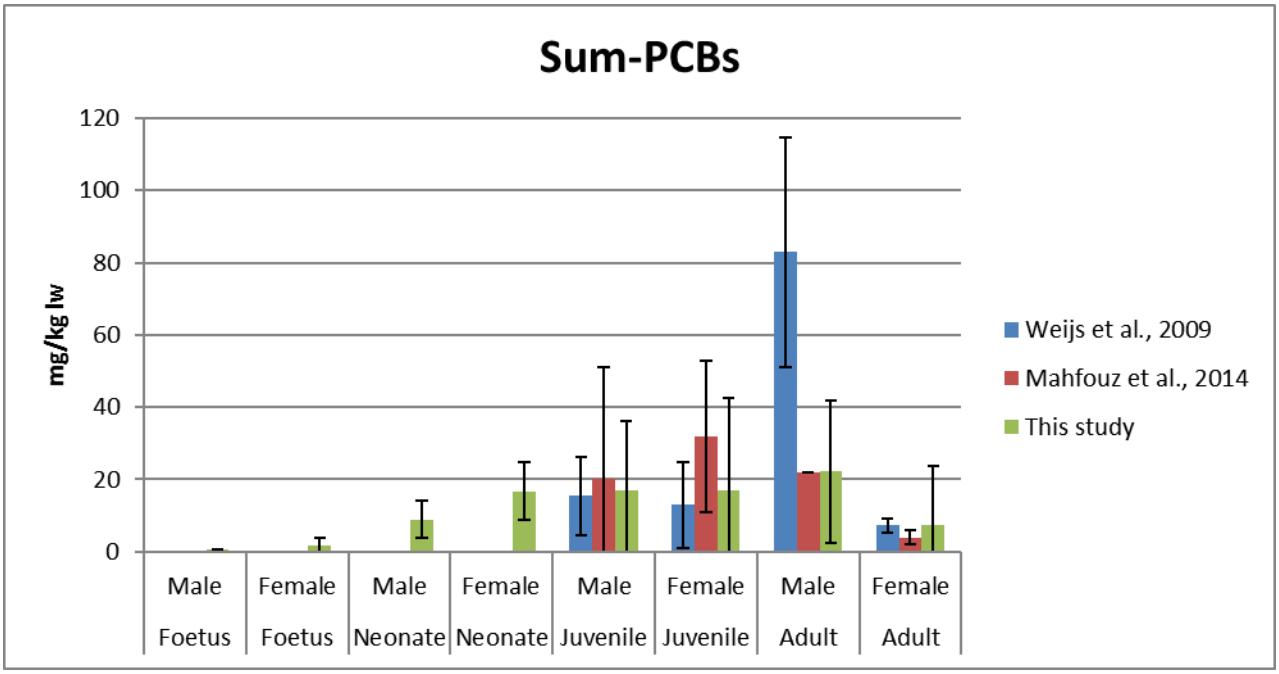

Figure 2 *Sum-PCB concentrations (in mg/kg lw) in blubber samples of different age groups of harbour porpoises of the North Sea (based on lipid weight). See Table 4 for locations of each group.*

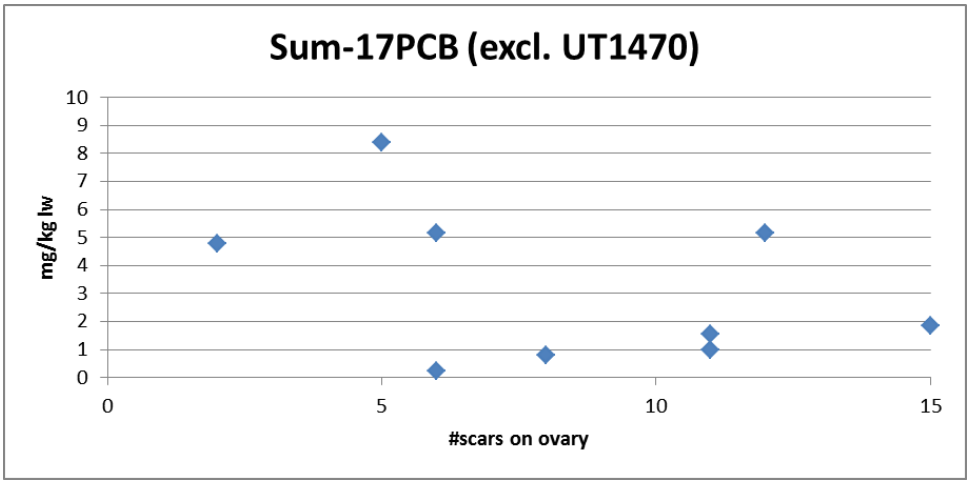

Figure 3 *Sum-PCB concentrations (in mg/kg lw) in a selection of blubber samples in relation to the number of ovarian scars of female harbour porpoises, beached along the Dutch coast (based on lipid weight). The safe level for Sum-PCBs is set at 9 mg/kg lipid weight (Jepson e.a., 2016). Sample UT1470 with deviating high PCB concentrations was omitted from this figure to be able to zoom in on the other scar-PCB relations.*

It is assumed that the more successful gestations an adult female harbour porpoise has, the higher the chance is that she offloaded lipophilic chemicals to her offspring through lactation (Murphy et al., 2015). The number of ovary scars reflects the number of ovulations that a female has had after reaching sexual maturity at an age of 3-4 years. The optimal calving time in the southern North Sea is believed to be May-July and therefore the fertility period is autumn. If reproduction is successful immediately, one ovulation is needed to become pregnant, resulting in one corpora scar on a female's ovary. A maximum number of ovulations of 3-4 per year is expected and when fertilisation is unsuccessful, the animal will be resting until the next fertility period the following year, in order to give birth in summer (pers.comm. Sinead Murphy). The number of ovary scars in relation to age is therefore used as an estimation for potential successful gestations. A high number of ovary scars in a relatively young animal is seen as a sign of unsuccessful reproduction, whilst an equal number of scars per year of age after sexual maturity suggests a yearly gestation.

When comparing the number of ovary scars with Sum-17PCB concentrations, concentrations decrease in general with increasing number of scars on ovary (Figure 3). This supports the theory that successful reproduction results in offloading of PCB contamination in adult females.

An exception was harbour porpoise UT1470 containing a Sum-17PCB concentration of 40.3 mg/kg lw at 15 ovary scars (not included in figure 3) with an approximate age of 7 years (see Annex A). This means that harbour porpoise UT1470 had a maximum number of ovulations per year to get to a total of 15 ovulations within this time frame. With 15 ovulations and no signs of PCB offloading, this may point at unsuccessful reproduction for this individual. This is supported by Murphy et al. (2015) who states that female harbour porpoises with a pollutant burden of >11 mg/kg lw (for Sum-25PCBs) may have never successfully offloaded their pollutant burden via gestation or lactation.

4.1.2 Comparison to international standards

PCB levels in 42 of the 91 samples of harbour porpoises beached along the Dutch coast were higher than the threshold level for toxicity of 9.0 mg/kg lw for onset of physiological endpoints in marine mammals (Jepson et al., 2016) (Figure 4). PCB levels in foetuses did not exceed this threshold level, suggesting no significant offloading in situ. However 60% of the neonates, 62% of the juveniles and 27% of the adults had PCB levels higher than this threshold level. When taking into account the sex of the porpoises, percentages of males porpoises exceeding the threshold were 50%, 65% and 85% for neonates, juveniles and adults respectively. For females this was 80%, 43% and 0% respectively.

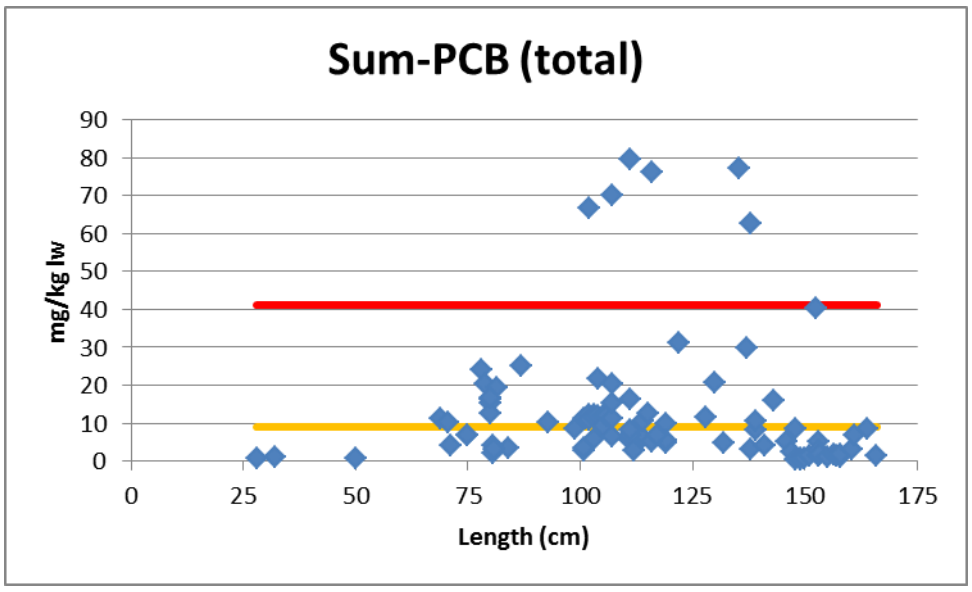

Figure 4 *PCB concentrations in blubber samples (in mg/kg lw) in relation to body length of harbour porpoises, beached along the Dutch coast (based on lipid weight) and threshold levels for toxicity of 9.0 mg/kg lw (for onset of physiological endpoints in marine mammals; yellow line) and 41 mg/kg lw (for reproductive impairment in seals; red line)(Jepson et al., 2016). See Annex H for number of samples per age group and sex.*

Additionally a threshold of 41 mg/kg lw Sum-PCB was introduced for reproductive impairment in marine mammals by Jepson et al. (2016). Six of the 91 harbour porpoise samples contained PCB levels exceeding this threshold. These composed of juveniles and adults, both males and females; all in a poor nutritional status (NCC>4). Emaciation may therefore increase PCB concentrations and therefore potentially enhance PCB effects of PCBs, and at the same time high PCB concentrations may affect the immune system and therefore nutritional status.

4.2 Polybrominated Diphenyl Ethers (PBDEs)

4.2.1 Levels in harbour porpoises

Sum-PBDE levels in blubber samples of harbour porpoises beached along the Dutch coastline between 2006-2016 were 0.002 – 2.000 mg/kg ww (Table 3) and 0.002 – 2.170 mg/kg lw (Table 4). This is a factor 10-30 lower than PCB levels in harbour porpoises. PBDE levels correspond with earlier published levels of PBDEs in harbour porpoises in the UK (0.06 – 1.04 mg/kg lw; Law et al., 2013), in the (southern) North Sea (0.76 mg/kg lw; Weijs et al., 2009)(0.09 – 0.38 mg/kg lw; Weijs et al., 2010)(1.89 mg/kg lw; Mahfouz et al., 2014), in Iceland (0.067 – 0.098 mg/kg lw; Rotander et al., 2012) and in Norway (0.071 – 0.540 mg/kg lw; Rotander et al., 2012).

The NCC of the beached porpoises influenced the PBDE concentrations in blubber with higher PBDE concentrations in porpoises with a poor nutritional status (NCC>4) and lower concentrations in individuals with a good to moderate nutritional status (NCC<4)(see Annex B). For a better comparison between age groups, samples with an NCC>4 were therefore omitted (see figure 5).

Accumulation of PBDEs in harbour porpoises followed the same patterns as in PCBs. Statistical analysis showed that for PBDEs there is not a significant interaction between age group and sex (see Annex G). This means that levels in harbour porpoises could be tested per sex and per age groups. Foetus samples had significantly lower PBDE levels than neonates, juveniles and adults (figure 5 and Annex G). Neonates accumulate PBDEs via lactation and this is further increased in juveniles, presumably via intake of food. In adult females, PBDE concentrations decrease. However, also larger and older adult males seemed to be able to decrease PBDE levels. This may imply that food intake is less of an important source for PBDEs for adults. Additionally, a less clear relation than for PCBs can be seen

between number of ovary scars and PBDE levels in adult female harbour porpoise (Annex B). This may imply that other routes of elimination may play a role for PBDEs besides offloading via milk. Again, harbour porpoise UT1470 showed a deviating high Sum-PBDE concentration pointing at a potential reproduction failure in this individual.

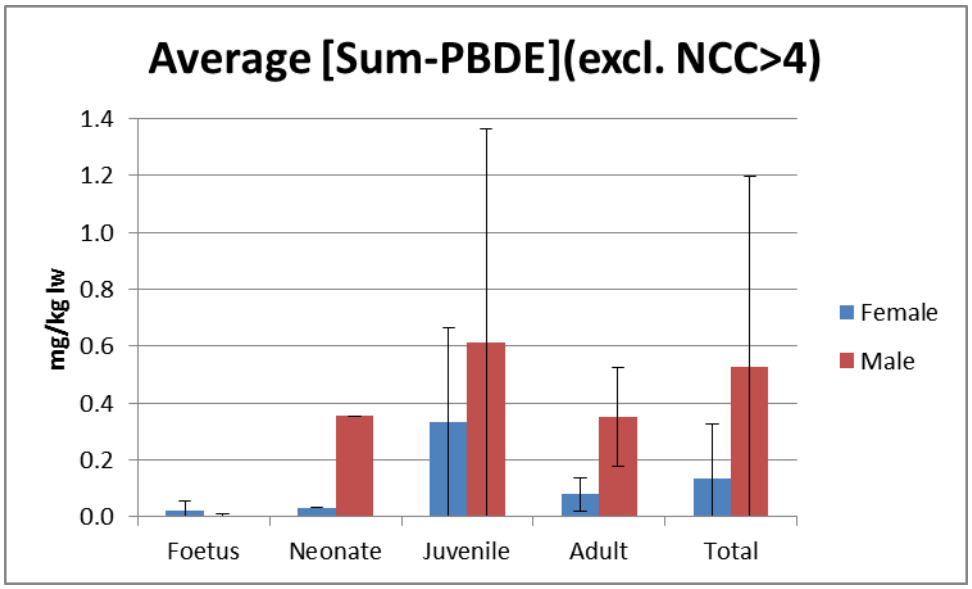

Figure 5 *Sum-6PBDE concentrations (in mg/kg lw) in a selection of blubber samples compared for all age classes of harbour porpoises, beached along the Dutch coast (based on lipid weight). Results of samples of harbour porpoises with a NCC>4 were excluded. Figure with all PBDE data incl. NCC>4 can be found in Annex B. See Annex H for number of samples per age group and sex.*

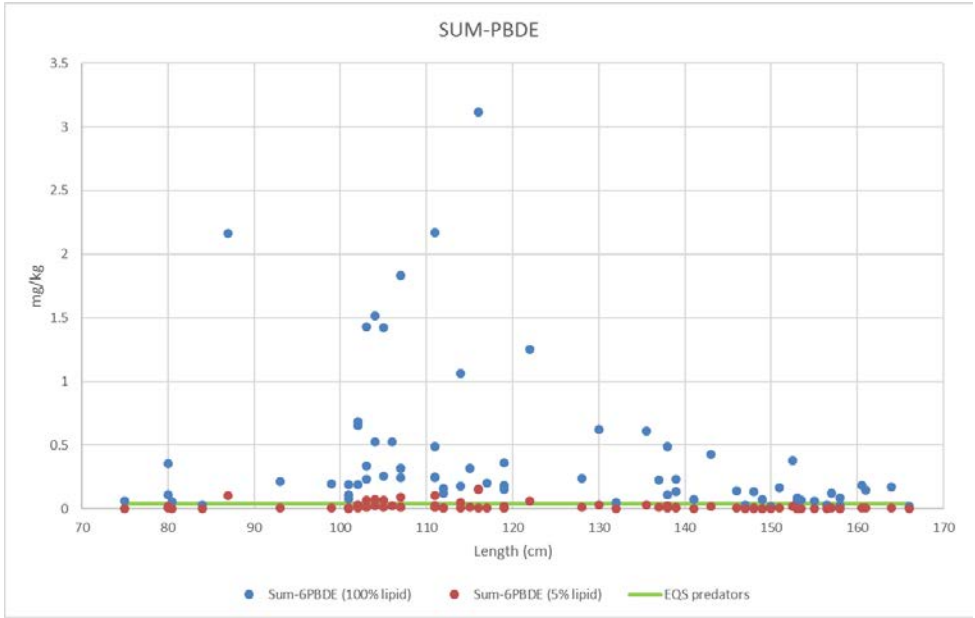

Figure 6 *Sum-6PBDE concentrations (in mg/kg lw) in blubber samples in relation to body length of harbour porpoises, beached along the Dutch coast (based on lipid weight) and the EQS threshold levels for toxicity of 9.0 mg/kg lw (for onset of physiological endpoints in marine mammals) and 41 mg/kg lw (for reproductive impairment in seals)(Jepson et al., 2016). Blue dots indicate concentrations based on 100% lipid, red dots are concentrations calculated on 5% lipid. See Annex H for number of samples per age group and sex.*

4.2.2 Comparison to international standards

The EQS Biota for Brominated diphenylethers is 0.0085 µg/kg wet weight (EC, 2013). The range of concentrations in analysed harbour porpoises were 2-2000 µg/kg wet weight. This is well above the EQS for PBDEs.

These levels were analysed in (pure) blubber samples (close to 100% lipid) of a toppredator (high in the food web). The EQS is however meant for fish. Fish samples often contain lipid levels of 5% in a wet weight sample. When the PBDE concentrations in harbour porpoises were calculated on a 5% lipid weight, this resulted in Sum-PBDE levels of 0.1 – 156 µg/kg 5% lipid weight. This was still more than a factor 10 above the EQS.

The EQS also mentions the threshold of 44 µg/kg wet weight for secondary poisoning of predators. 66 of the 78 samples had Sum-PBDE levels above this threshold (when based on 100% lipid) (see blue dots in figure 6). However, it is not clear if this EQS for secondary poisoning of predators is also based on 5% lipid. All 4 foetus samples were below the threshold.

Environmental Quality Standard for PBDEs
According to the European Directive 2013/39/EU the following environmental quality standards (EQS) are applied for biota, i.e. fish: 0,0085 µg/kg for the sum of PBDEs. EQS is related to fresh weight. According to actual discussions concerning the use of 5% fat-normalised concentrations, the corresponding EQS is presented on lipid base additionally, considering a lipid content of 5% is 0,17 µg/kg fat. With regard to PBDEs the EQS of 0,0085 µg/kg is related to the protection of human health, while the EQS concerning the secondary poisoning of predators is 44 µg/kg (EQSSP) 12:
12. Sub-Group on Review of the Priority Substances List: PolyBrominated Diphenyl Ethers (BDEs), Dossier 2011. For the group of priority substances covered by brominated diphenylethers (No 5), the EQS refers to the sum of the concentrations of congener numbers 28, 47, 99, 100, 153 and 154. https://circabc.europa.eu/webdav/CircaBC/env/wfd/Library/framework_directive/thematic_documents/priority_substances/supporting_substances/eqs_dossiers/PBDE%20EQS%20dossier%202011.pdf accessed 15 May 2015)

4.3 Hexachlorobutadiene (HCBD)

4.3.1 Levels in harbour porpoises

All analysed samples of harbour porpoises beached along the Dutch coast, contained HCBD levels below detection limit.

4.3.2 Comparison to international standards

The EQS in biota for Hexachloro- butadiene (HCBD) is 55 µg/kg wet weight (EC, 2011). As all samples contained levels below the detection limit, no samples were above the EQS.

Environmental Quality Standard for HCBD
Unless otherwise indicated, the biota EQS relate to fish. An alternative biota taxon, or another matrix, may be monitored instead, as long as the EQS applied provides an equivalent level of protection.

4.4 Hexachlorobenzene (HCB)

4.4.1 Levels in harbour porpoises

HCB levels in blubber samples of adult harbour porpoises beached along the Dutch coastline between 2006-2016 were 0.01 – 0.56 mg/kg ww (Table 3) and 0.015 - 0.586 mg/kg lw (Table 4). This is slightly lower than the reported PBDE concentrations in these harbour porpoises and corresponds with earlier published levels of HCB in harbour porpoises of the southern North Sea (0.09 – 0.35 mg/kg lw; Weijs et al., 2010)(0.01 – 1.50 mg/kg lw; Imazaki et al., 2015).

The NCC of the beached porpoises influenced the HCB concentrations in blubber slightly. Highest HCB levels were found in porpoises with a moderate to poor nutritional status (NCC>3)(see Annex C). As the effects of NCC is not more than a factor 2 and only 18 samples of adult harbour porpoises were analysed for HCB levels over the years, all samples were kept for comparisons.

HCB concentrations were only analysed in these harbour porpoise samples in 2016, and not in previous years. The dataset for harbour porpoises beached along the Dutch coast is therefore very small for HCB. HCB concentrations in males were a factor 3 higher than those in adult females (Figure 7). This also suggests offloading of HCB through lactation.

4.4.2 Comparison to international standards

The EQS Biota for Hexachlorobenzene (HCB) in biota is 10 µg/kg wet weight. Samples of harbour porpoises contained HCB levels of 13-560 µg/kg wet weight (figure 8). These are a factor of 1 - 50 above the EQS biota. However, unless otherwise indicated, the biota EQS relates to fish. An alternative biota taxon, or another matrix, may be monitored instead, as long as the EQS applied provides an equivalent level of protection.

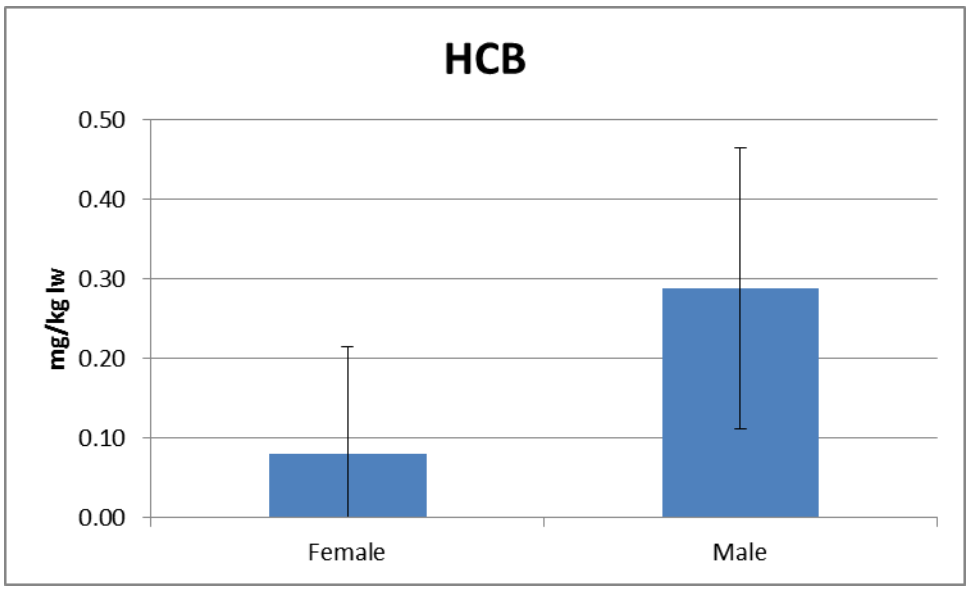

Figure 7 *HCB concentrations (in mg/kg lw) in blubber samples of adult female and male harbour porpoises, beached along the Dutch coast (based on lipid weight). See Annex H for number of samples per age group and sex.*

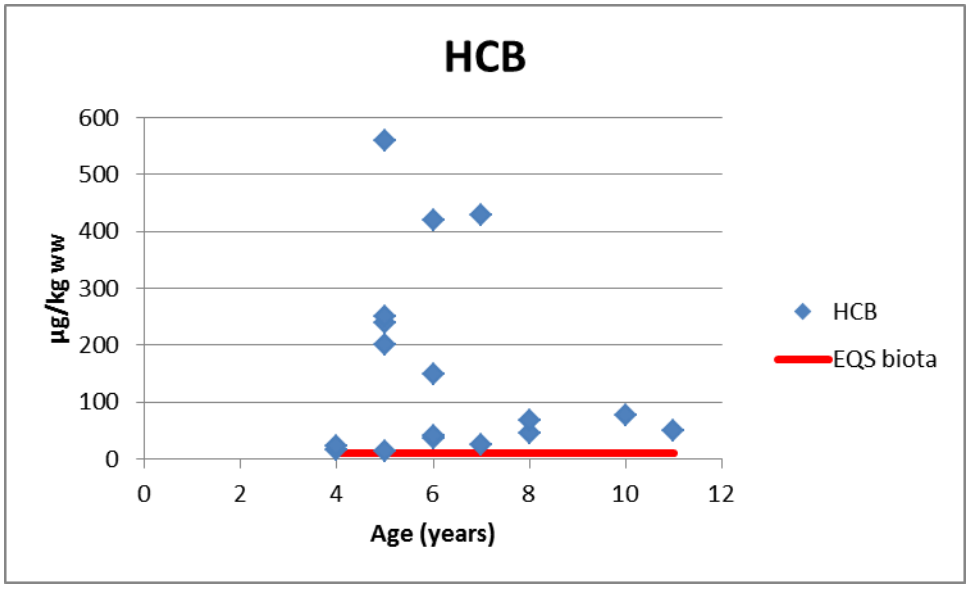

Figure 8 *HCB concentrations (in mg/kg ww) in blubber samples of female harbour porpoises in relation to age, beached along the Dutch coast and the EQS of 10 µg/kg for biota (based on wet weight). See Annex H for number of samples per age group and sex.*

4.5 Dioxin-like PCBs (dl-PCBs)

4.5.1 Levels in harbour porpoises

PCBs consist of 209 different compounds (congeners). Of these PCBs congeners, coplanar PCBs are most toxic possessing a dioxin-like toxicity (dioxin-like PCBs - dl-PCBs). This type of toxicity can be calculated using a Toxic Equivalent Factor (TEF) for each of the dl-PCBs. To derive the Toxic Equivalent for dioxins and dioxin-like compounds (TEQ) the concentration of each dl-PCB is multiplied with a specific TEF for each of the dl-like PCBs.

Dioxin-like compounds accumulate in food webs. However, the composition may change from low to high in the food web. Low in the food web (such as sediments) the mixture will compose mainly of dioxins and furans whereas higher up in the food web dioxin-like PCBs are more prone to accumulate than dioxins and furans. Therefore, in toppredators, such as harbour porpoises, most of the TEQ will consist of dl-PCBs.

In this study dl-PCBs were analysed in samples of adult harbour porpoises beached along the Dutch coast between 2014-2016. Sum-dl-PCB levels in blubber samples of these porpoises were 0.04 – 1.46 mg/kg ww (Table 3) and 0.05 – 1.64 mg/kg lw (Table 4). This is only a small percentage of the Sum-PCB concentrations found in harbour porpoises (2.5% of the total Sum-PCBs based on average concentrations). Concentrations were in the same order of magnitude as Sum-PBDE levels in blubber of harbour porpoises. When based on TEQ, Sum-dl-PCB levels were 1 - 86 ng/kg TEQ ww (Table 3) and 3 - 97 ng/kg TEQ lw (Table 4).

The NCC of the beached porpoises may have slightly influenced Sum-dl-PCB concentrations in blubber. The highest levels of Sum-dl-PCBs were found in porpoises with a poor nutritional status (NCC>4)(see Annex D).

Only samples of adult harbour porpoises were analysed for levels of Sum-dl-PCBs. Sum-dl-PCBs concentrations in males were slightly higher than those in adult females (Figure 9), although one high value in adult male samples influenced this greatly. When omitting this high value, levels between males and females were in the same order of magnitude (see Annex D).

Number of ovary scars was not correlated as much to Sum-dl-PCB concentrations when compared to the other PCB congeners (see figure 3 and Annex D).

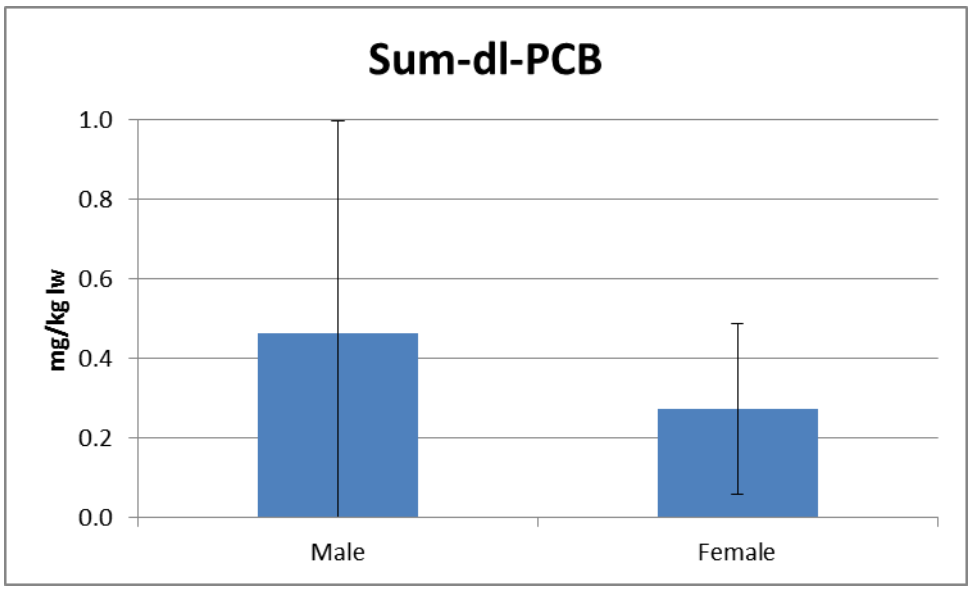

Figure 9 *Dioxin-like PCB concentrations (in mg/kg lw) in blubber samples of adult female and male harbour porpoises, beached along the Dutch coast (based on lipid weight). See Annex H for number of samples per age group and sex.*

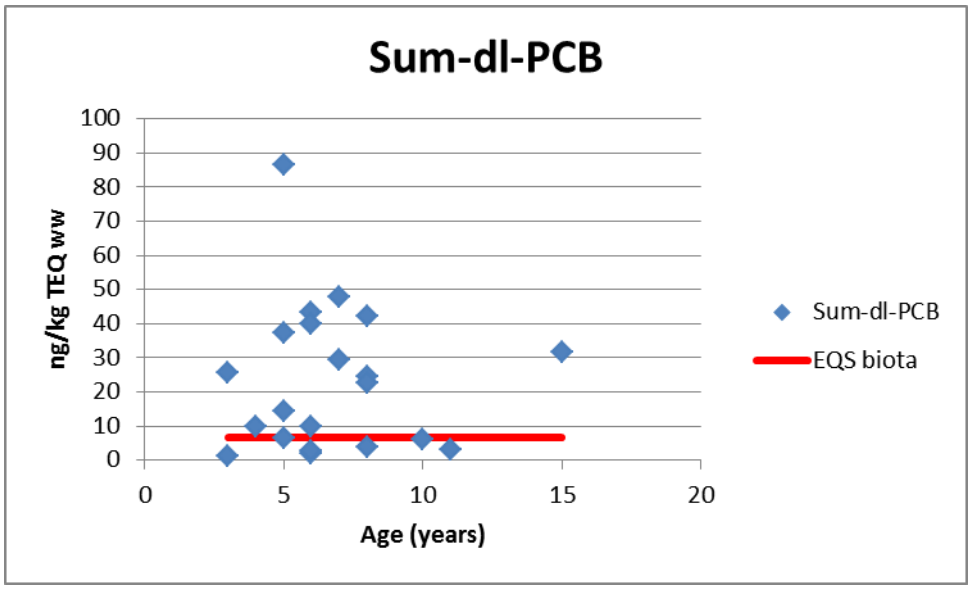

Figure 10 *Dioxin-like PCB concentrations (in ng/kg TEQ ww) in blubber samples of adult harbour porpoises in relation to age, beached along the Dutch coast (based on wet weight) and the EQS of 6.5 ng/kg TEQ wet weight for biota. See Annex H for number of samples per age group and sex.*

4.5.2 Comparison to international standards

The EQS Biota for dioxins and dioxin-like compounds (including dl-PCBs) is 6.5 ng/kg TEQ wet weight. Samples of harbour porpoises had dl-PCB levels of 1 - 86 ng/kg TEQ wet weight. 14 of 21 samples were above the EQS (excluding one samples exactly at the level of the EQS biota) (figure 10). However, the EQS for dioxins and dioxin-like compounds relates to fish, crustaceans and molluscs, and constructed to protect set safe levels for these compounds in foodstuffs.

Environmental Quality Standard for dioxin-like PCBs

Unless otherwise indicated, the biota EQS relate to fish. An alternative biota taxon, or another matrix, may be monitored instead, as long as the EQS applied provides an equivalent level of protection. For substance number 37 (Dioxins and dioxin-like compounds), the biota EQS relates to fish, crustaceans and molluscs, in line with section 5.3 of the Annex to Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs (OJ L 320, 3.12.2011, p. 18).

4.6 PerFluorinated compounds (PFCs)

4.6.1 Levels in harbour porpoises

Sum-PFC levels in liver samples of adult harbour porpoises beached along the Dutch coastline between 2006-2016 were 0.05 – 3.0 mg/kg ww (Table 3) and 0.17 – 10.6 mg/kg dw (dry weight). Comparison with other contaminants is not possible as only PFCs were analysed in liver samples as PFCs tend to accumulate in liver (and blood) instead of in blubber (Ylinen & Auriola, 1990). Most of the Sum-PFC concentrations in these livers consisted of PFOS, with levels of 0.05 – 3.0 mg/kg ww (Table 3) and 0.16 – 10.2 mg/kg dw. PFOS levels correspond well with levels found in other parts of the North Sea and earlier studies (table 6). Only concentrations in West Iceland are lower than reported for the North Sea.

The NCC of the beached porpoises influenced PFC concentrations in liver. But in contrast with the lipophilic contaminants (PCBs, HCB), the highest PFC levels were found in porpoises with a good to moderate nutritional status (NCC<4) and lower PFC levels were found in porpoises with a poor nutritional status (NCC>4) (see Annex C).

Highest PFC/PFOS concentrations were observed in liver samples of neonate and juvenile harbour porpoises, between body lengths of 75-125 cm (Figure 11). Concentrations in adult males were in the same order of magnitude as concentrations in adult females and lower than those found in neonates and juveniles (Figure 12).

The number of ovary scars seemed slightly correlated to PFC concentrations with higher PFC concentrations with higher number of ovary scars (see figure Annex E), although the reason for this is not known.

Table 6

PFOS levels in liver samples of harbour porpoises of the northeast Atlantic.

Compound	n		Year	Location	Range	Reference
PFOS	62	mg/kg ww	1980-2005	North Sea - DK	0.05 – 1.70	Galatius et al., 2011
PFOS	37	mg/kg ww	1991-2008	Baltic Sea	0.16 – 2.43	Huber et al., 2012
PFOS	24	mg/kg ww	1991-2008	North Sea	0.20 – 2.40	Huber et al., 2012
PFOS	6	mg/kg ww	1992-1997	West Iceland	0.02 – 0.06	Rotander et al., 2012a
PFOS	11	mg/kg ww	1999-2002	North Sea - DK	0.09 – 0.53	Galatius et al., 2013
PFOS	59	mg/kg ww	2006-2016	North Sea – NL	0.05 – 3.00	This study

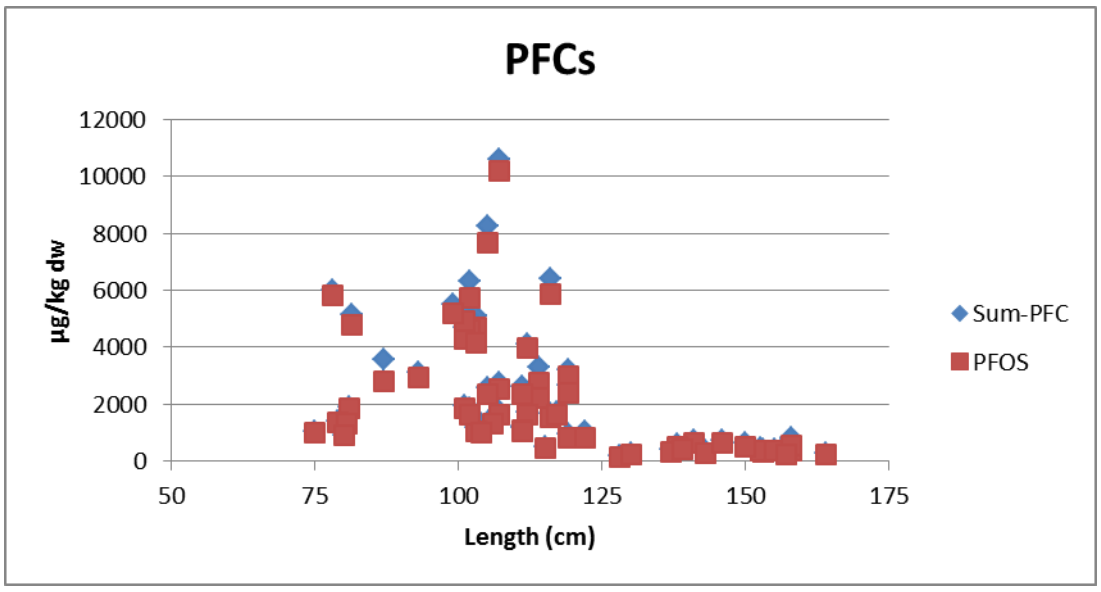

Figure 11 *PFC concentrations (in µg/kg dw) in liver samples in relation to body length of harbour porpoises, beached along the Dutch coast (based on dry weight). See Annex H for number of samples per age group and sex.*

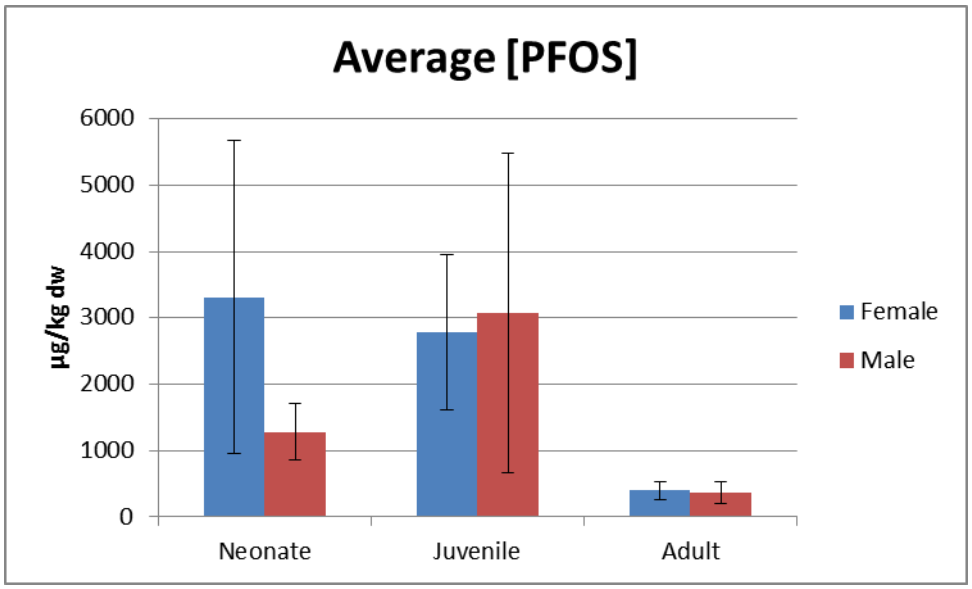

Figure 12 *Average PFOS concentrations (in µg/kg dw) in liver samples of three age classes of harbour porpoises, beached along the Dutch coast (based on dry weight). See Annex H for number of samples per age group and sex.*

4.6.2 Comparison to international standards

The EQS Biota for PFOS is 9,1 µg/kg wet weight. Samples of harbour porpoises had PFOS levels of 50 – 3000 µg/kg wet weight (figure 13). These are all above the EQS biota. Also here, the EQS is related to fish and not higher trophic levels.

Environmental Quality Standard for PFOS
Perfluorooctane sulfonic acid and its derivatives (PFOS): 9.1 µg/kg wet weight
Unless otherwise indicated, the biota EQS relate to fish. An alternative biota taxon, or another matrix, may be monitored instead, as long as the EQS applied provides an equivalent level of protection.

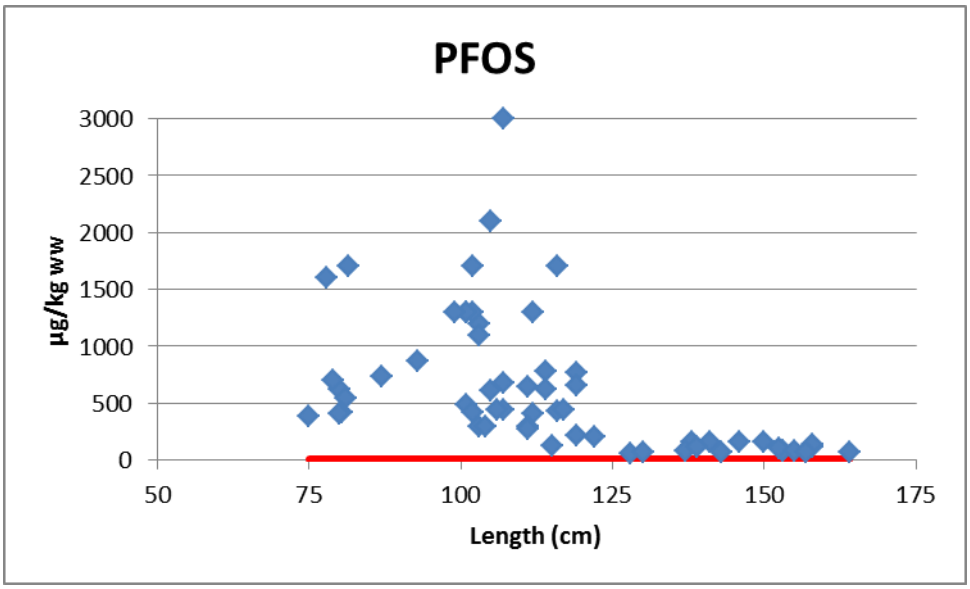

Figure 13 *PFOS concentrations (in µg/kg ww) in liver samples in relation to body length of harbour porpoises, beached along the Dutch coast (based on wet weight) and the EQS of 9,1 µg/kg wet weight for biota. See Annex H for number of samples per age group and sex.*

5 Conclusions and recommendations

5.1 Conclusions

Sub questions as addressed in this study can be answered as following based on the obtained results:

1) What are contaminant levels in adult harbour porpoises beached along the Dutch shorelines?
PCBs (including dioxin-like PCBs), PBDEs, HCBD and HCB were analysed in blubber samples of harbour porpoises beached along the Dutch coast line. Data are expressed as wet weight (ww) or lipid weight (lw) due to the lipophilic characteristics of these compounds. PCBs were found in highest levels of these contaminants. Sum-PCB levels in blubber were 0.02 – 56.1 mg/kg ww and 0.2 – 80 mg/kg lw. Other contaminants were found at considerably lower concentrations than PCBs. Sum-PBDE levels in blubber samples were 0.002 – 2.000 mg/kg ww and 0.002 – 2.170 mg/kg lw. HCB levels in blubber samples were 0.01 – 0.56 mg/kg ww and 0.015 - 0.586 mg/kg lw. The sum of dioxin-like PCBs in blubber samples were 0.04 – 1.46 mg/kg ww and 0.05 – 1.64 mg/kg lw. This is only a small percentage of the Sum-PCB concentrations found in harbour porpoises (2.5% of the total Sum-PCBs based on average concentrations). When based on TEQ, Sum-dl-PCB levels were 1 - 86 ng/kg TEQ ww and 3 - 97 ng/kg TEQ lw. HCBD was not found in levels above the detection limits in the analysed samples.

PFCs were analysed in liver samples. Data are expressed as wet weight (ww) or dry weight (dw) due to their protein-binding characteristics. Sum-PFC levels were 0.05 – 3.0 mg/kg ww and 0.17 – 10.6 mg/kg dw (dry weight).

Contaminant concentrations as measured in this study were of the same order of magnitude as earlier published levels in harbour porpoises in the UK and North Sea.

Of three out of four samples with the highest PCB concentrations (based on lipid weight), also highest PBDE concentrations were found (up to top 4, based on lipid weight) and in one case also highest PFOS concentrations (top 3, based on dry weight). A further multivariate analysis can be conducted to assess potential relations between contaminant groups and concentrations.

2) Do age and length influence contaminant concentrations in harbour porpoises beached along the Dutch shorelines?
Both age and length influence contaminant concentrations in harbour porpoises. Levels are relatively low in foetuses of harbour porpoises, but increase during lactation and the juvenile stage. PCBs and HCB tend to offload during pregnancy and especially lactation of adult females, decreasing the concentrations in blubber of these females but increasing the levels in calves. Both PBDEs and PFCs showed lower concentrations after the juvenile stage, whereas concentrations remained high in adult males for both PCBs and HCB.

3) Does number of ovulations influence contaminant concentrations in harbour porpoises beached along the Dutch shorelines?
Both for PCBs and HCB an increase in number of ovulations (corresponding with their age) was correlated with a decrease in contaminant concentrations. This implies that females with a higher number of pregnancies were able to offload these contaminants and had a successful reproduction and lactation period. This correlation was not so obvious for PBDEs, and the low number of data for PFCs may point at an increase in liver concentrations with increasing number of ovulations.

One adult females showed deviating high concentrations of PCBs and PBDEs in combination with a high number of ovulations in relation to her age. Total PCB concentrations were higher than levels

reported for reproductive impairment. The fact that this female was not able to offload PCBs despite a high number of ovulations may point at reproduction failure.

4) Do contaminants form a potential pressure on the harbour porpoise population in the North Sea?

This report presents a first overview of contaminant levels in all age classes of harbour porpoises beached along the Dutch coast. Both neonates (new-born calves) and juveniles obtain high levels of different types of lipophilic contaminant groups during their early life stage, up to levels that may exert physiological or reproductive effects. Adult male porpoises accumulate highest PCB and HCB levels in their blubber relative to other age classes and the female sex. Adult female porpoises can offload their PCB and HCB burden via placenta transfer and especially through lactation.

Highest PCB concentrations were observed in adult male harbour porpoises. 85% of the adult males had PCB levels above the threshold for physiological effects in marine mammals (9 mg/kg lw), whereas one male had PCB levels above the threshold for reproductive impairment (41 mg/kg lw). Adult females had lower levels of PCBs. They seem to have offloaded their PCB concentrations via gestation and lactation, with the exception of one adult female with high PCB levels, above the threshold for reproductive impairment. This adult female had the highest number of ovulations but also deviating high levels of PCBs pointing at reproductive impairment which was supported by scientific literature.

The EQS for biota was often exceeded in both blubber (PBDEs, HCB) and liver (PFCs) samples of the analysed harbour porpoises. However, the EQS in biota is related to fish or in some cases shell fish, whereas harbour porpoises are much higher up in the food web. The contaminant groups analysed in this study are all prone to accumulate in food webs. To be able to make a better comparison with EQS for fish, a biomagnification factor may be taken into account to estimate an EQS for higher trophic levels based on the EQS for fish.

Emaciation affects contaminant concentrations in blubber (especially PCBs) as it concentrates contaminants when the blubber volume decreases. This may also result in a release of PCBs from the blubber storage back into the organism potentially affecting the health of the emaciated porpoise. At the same time increased PCB concentrations may affect the immune system of the porpoise, which may consequently lead to a decreased nutritional status.

This shows that both reproduction success and the immune system of harbour porpoises can be affected by contaminants, already in their early life stages. This may have consequences for the stability of the porpoise population in the southern North Sea. Further insight into the role of contaminants on the fitness of harbour porpoises is therefore urgently needed.

5.2 Recommendations

1. Estimate ESQ in biota for the level of harbour porpoises based on biomagnification factors for harbour porpoises consuming fish;
2. Further analyse pathology data in relation to contaminant and NCC data;
3. Further study the relation between ovulation scars, age, contaminant levels and other signs of reproduction failure in future samples (preferably in adult female with extreme high levels, such as UT1470)
4. Assess whether neonates are affected by high contaminant levels through comparison of contaminant data with pathological findings.

6 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2008 certified quality management system (certificate number: 187378-2015-AQ-NLD-RvA). This certificate is valid until 15 September 2018. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V.

Furthermore, the chemical laboratory at IJmuiden has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2017 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation. The chemical laboratory at IJmuiden has thus demonstrated its ability to provide valid results according a technically competent manner and to work according to the ISO 17025 standard. The scope (L097) of de accredited analytical methods can be found at the website of the Council for Accreditation (www.rva.nl).

On the basis of this accreditation, the quality characteristic Q is awarded to the results of those components which are incorporated in the scope, provided they comply with all quality requirements. The quality characteristic Q is stated in the tables with the results. If, the quality characteristic Q is not mentioned, the reason why is explained.

The quality of the test methods is ensured in various ways. The accuracy of the analysis is regularly assessed by participation in inter-laboratory performance studies including those organized by QUASIMEME. If no inter-laboratory study is available, a second-level control is performed. In addition, a first-level control is performed for each series of measurements.
In addition to the line controls the following general quality controls are carried out:

- Blank research.
- Recovery.
- Internal standard
- Injection standard.
- Sensitivity.

The above controls are described in Wageningen Marine Research working instruction ISW 2.10.2.105. If desired, information regarding the performance characteristics of the analytical methods is available at the chemical laboratory at IJmuiden.

If the quality cannot be guaranteed, appropriate measures are taken.

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Justification

Report C069/17
Project Number: 4315100055

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

Approved: Dr. D.M.E. Slijkerman
Researcher

Signature: 

Date: 29th of August 2017

Approved: Dr. J. Asjes
Manager Integration

Signature: 

Date: 29th of August 2017

Annex A PCB figures

This annex presents additional figures to the figures that are incorporated in the main text.

Nutritional status versus PCB levels

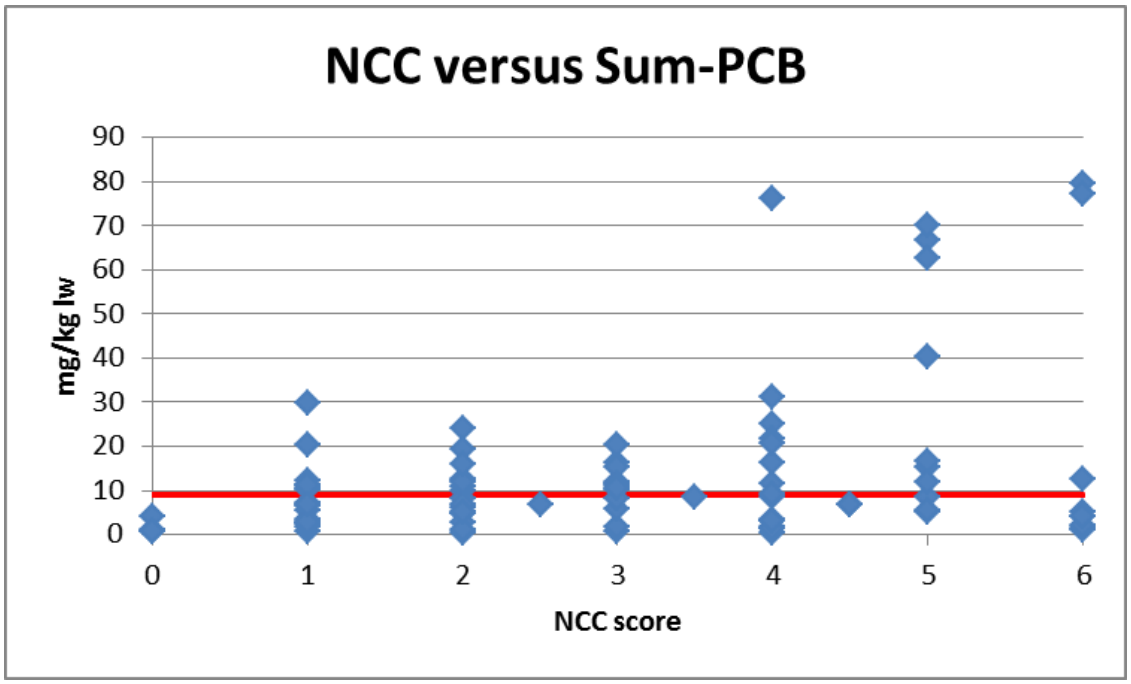

Figure A.1 *Sum-17PCB concentrations (in mg/kg lw) in blubber samples in relation to NCC score of harbour porpoises, beached along the Dutch coast (based on lipid weight). The safe level for Sum-PCBs is set at 9 mg/kg lipid weight (Jepson e.a., 2016).*

The nutritional status of a beached harbour porpoise greatly influences the concentration of lipophilic substances in blubber samples. Due to starvation PCB may become concentrated in blubber tissue (showing higher concentrations in emaciated porpoises than in non-emaciated porpoises) or redistributed within the body (Van den Heuvel-Greve et al., 2011).

NCC is a measure for nutritional status. The lower the NCC value the better the nutritional status of the harbour porpoise, whereas the higher the value the more emaciated the harbour porpoise. In figure A.1 it is shown that despite a general spread in contaminant levels, outliers of a sum-17PCB concentration of more than 40 mg/kg lipid weight are only occurring in samples from porpoises with an NCC of 4 or more. As NCC clearly affects PCB levels, NCC>4 have been excluded in occasions to obtain a better insight when comparing contaminant levels between age classes or sexes.

Age versus PCB levels

Age influences PCB concentrations in blubber with decreasing PCB levels with increasing age (figure A.2). However, only females seem to obtain lower levels later in life. After 4-5 years of age Sum-17PCB concentrations in females were below the threshold level for physiological effects of 9 mg/kg lipid weight (Jepson et al., 2016). This is due to the transfer of the contaminant load to their offspring via the placenta and through lactation (Van den Heuvel-Greve et al., 2016). Sum-17PCB levels in male harbour porpoises show an opposite trend. After the age of 5 all male individuals showed a contaminant load above the threshold level of 9 mg/kg lipid weight.

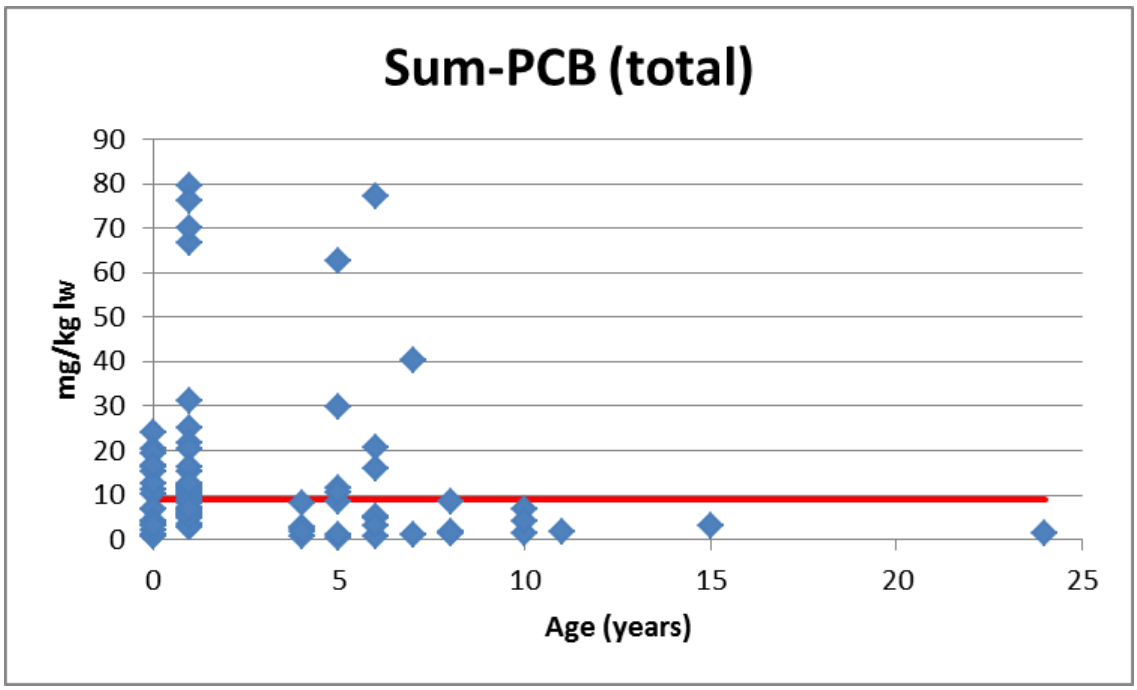

Figure A.2 *Sum-17PCB concentrations (in mg/kg lw) in blubber samples in relation to age of harbour porpoises, beached along the Dutch coast (based on lipid weight). The threshold level for Sum-PCBs is set at 9 mg/kg lipid weight (Jepson e.a., 2016).*

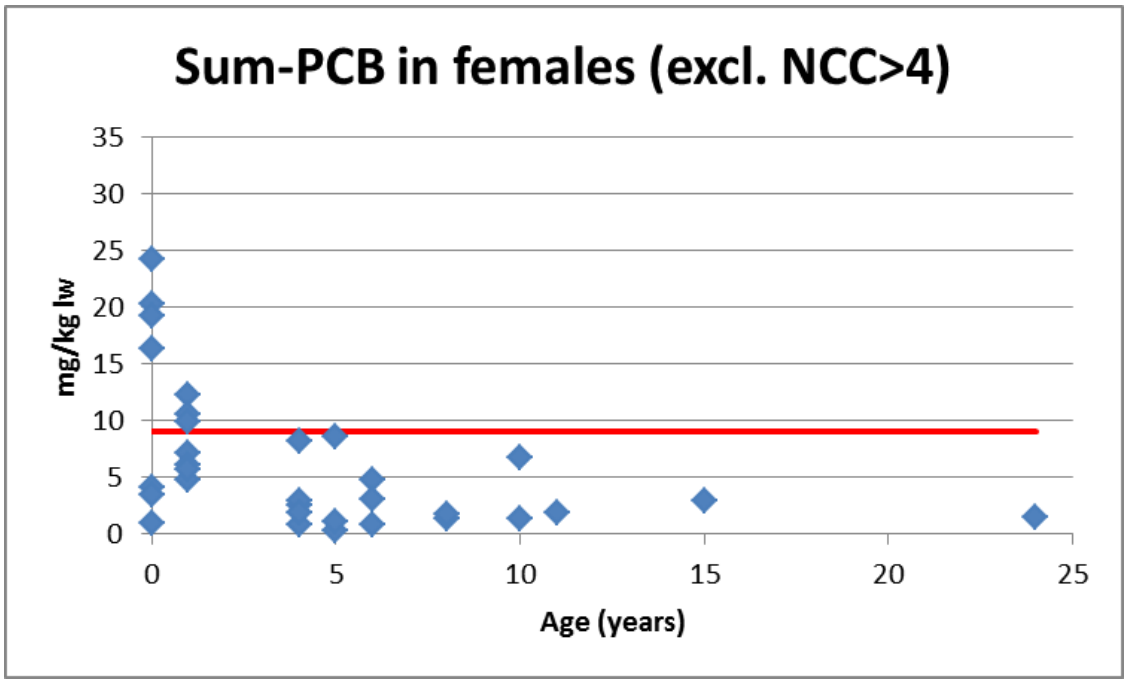

Figure A.3 *Sum-17PCB concentrations (in mg/kg lw) in blubber samples in relation to age of female harbour porpoises, beached along the Dutch coast (based on lipid weight). The threshold level for Sum-PCBs is set at 9 mg/kg lipid weight (Jepson e.a., 2016). Results of samples of harbour porpoises with a NCC>4 were excluded.*

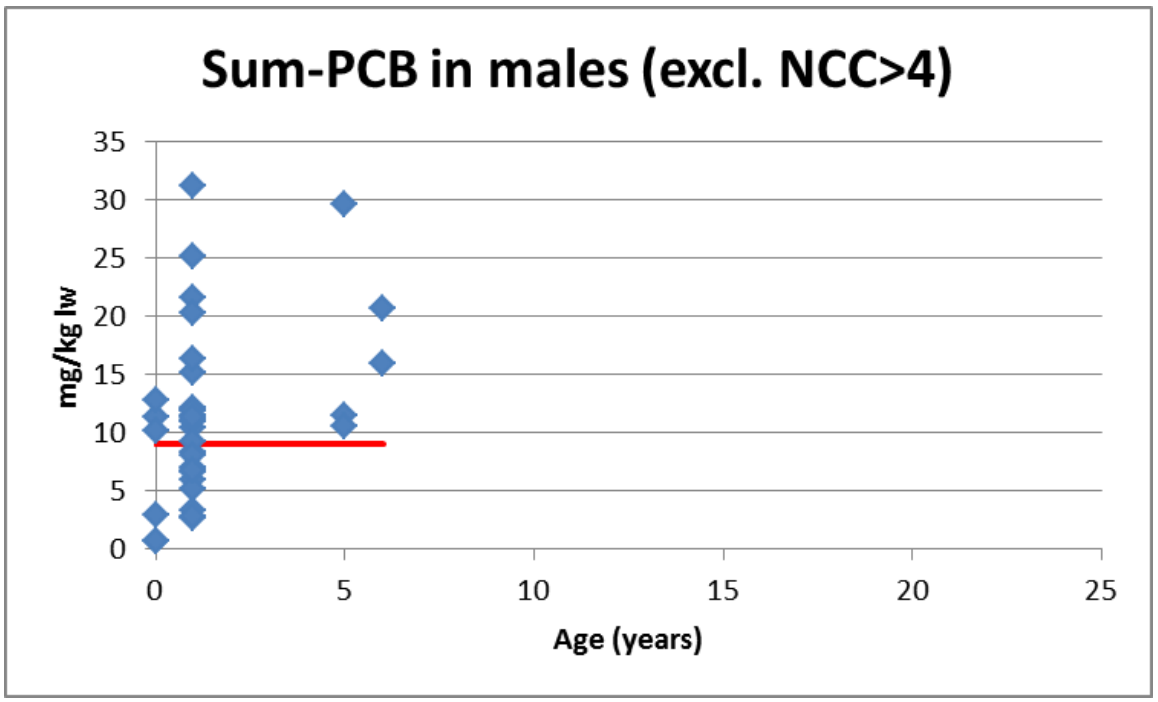

Figure A.4 *Sum-17PCB concentrations (in mg/kg lw) in blubber samples in relation to age of male harbour porpoises, beached along the Dutch coast (based on lipid weight). The threshold level for Sum-PCBs is set at 9 mg/kg lipid weight (Jepson e.a., 2016). Results of samples of harbour porpoises with a NCC>4 were excluded.*

Length versus PCB levels

When comparing length of the individuals with Sum-17PCB concentrations in blubber, an increase of PCB concentrations is seen with increasing length (Figure A.5). When only considering male harbour porpoises, PCB concentrations indeed increase with increasing body length (Figure A.7). However, when looking at females only Sum-17PCB concentrations decrease when females reach a body length of 125 cm or more (close to the length at which females obtain a reproductive state)(Figure A.6).

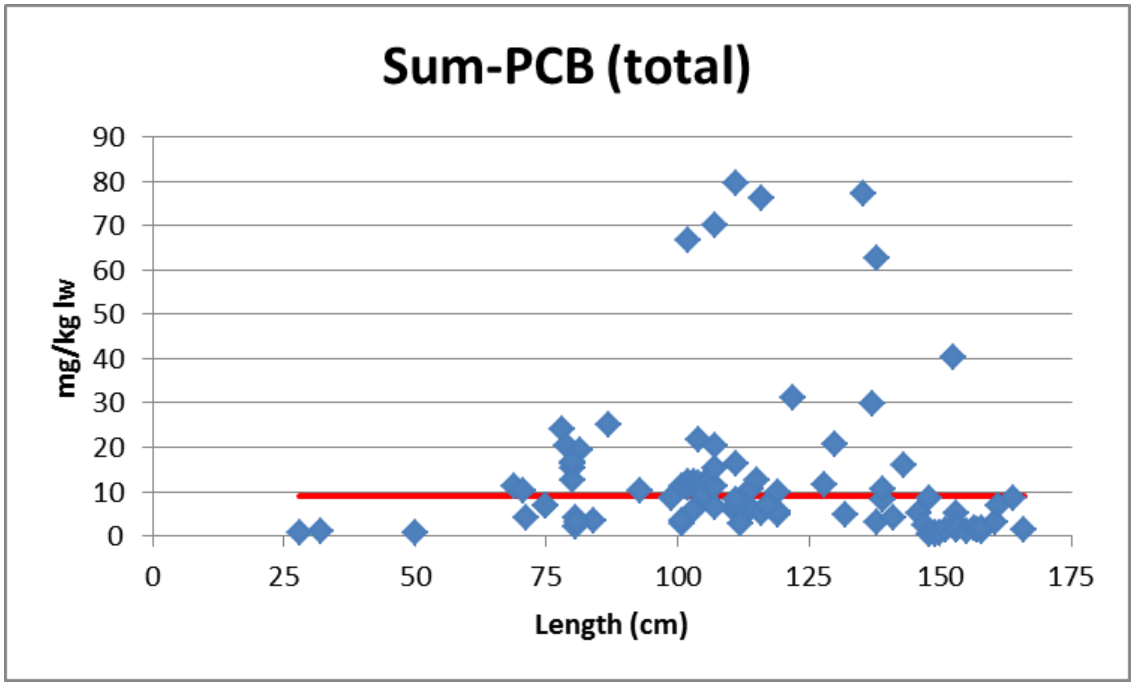

Figure A.5 *Sum-17PCB concentrations (in mg/kg lw) in blubber samples in relation to body length of harbour porpoises, beached along the Dutch coast (based on lipid weight). The safe level for Sum-PCBs is set at 9 mg/kg lipid weight (Jepson e.a., 2016).*

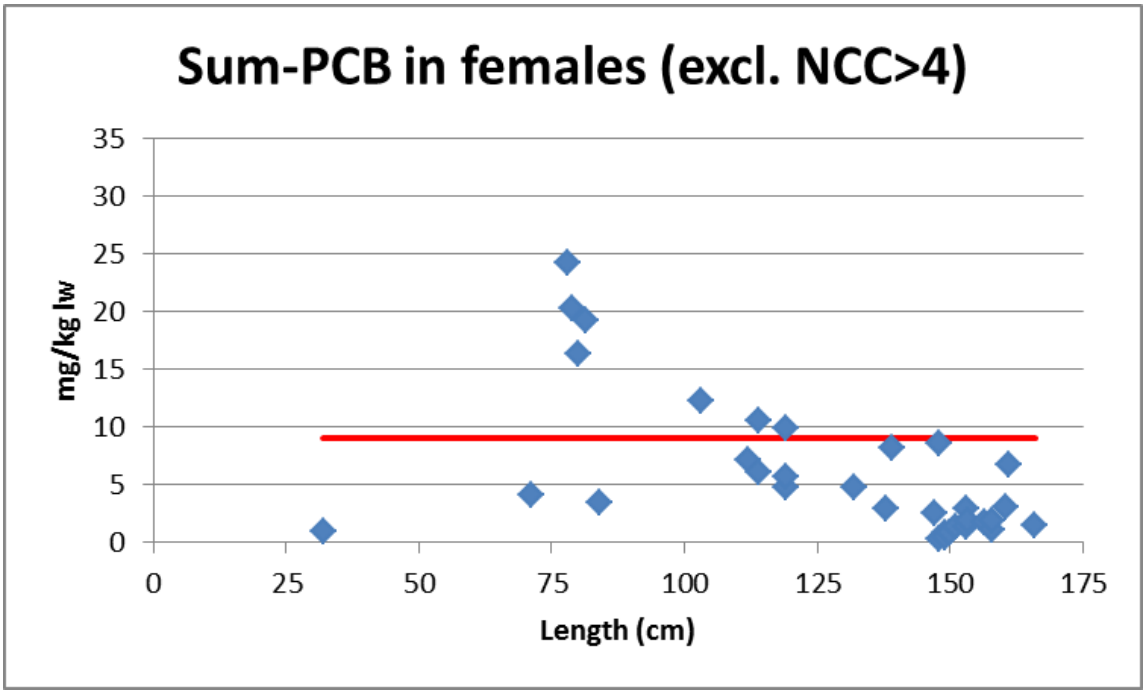

Figure A.6 *Sum-17PCB concentrations (in mg/kg lw) in blubber samples in relation to body length of female harbour porpoises, beached along the Dutch coast (based on lipid weight). The safe level for Sum-PCBs is set at 9 mg/kg lipid weight (Jepson e.a., 2016). Results of samples of harbour porpoises with a NCC>4 were excluded.*

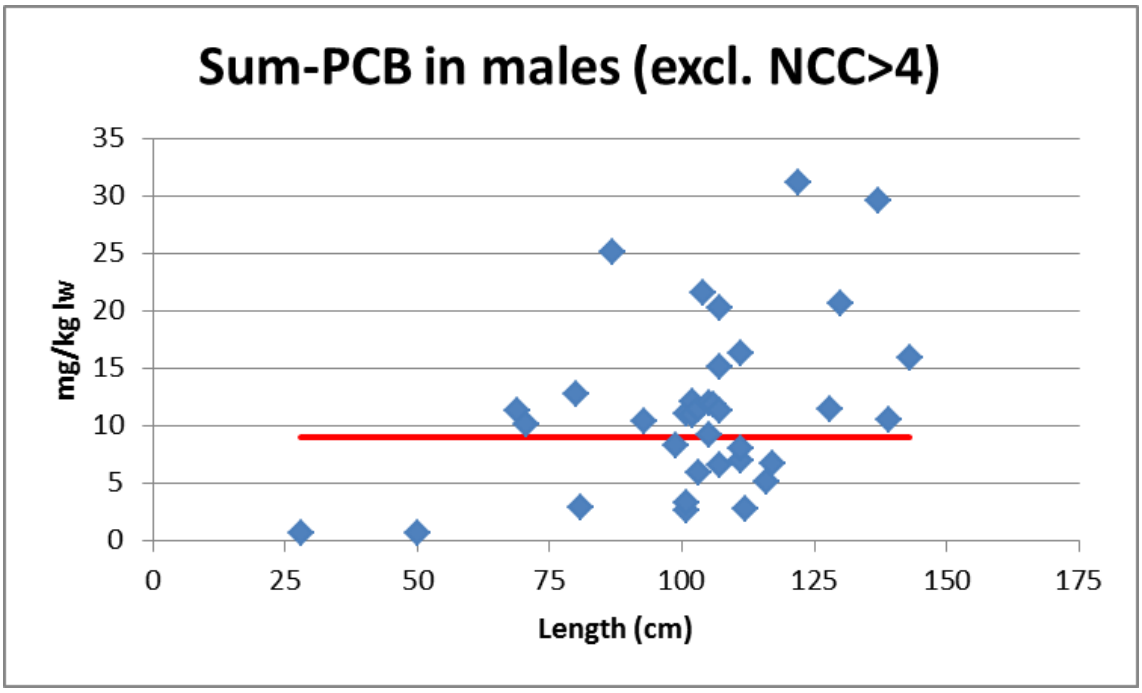

Figure A.7 *Sum-17PCB concentrations (in mg/kg lw) in blubber samples in relation to body length of male harbour porpoises, beached along the Dutch coast (based on lipid weight). The safe level for Sum-PCBs is set at 9 mg/kg lipid weight (Jepson e.a., 2016). Results of samples of harbour porpoises with a NCC>4 were excluded.*

Ovary scars

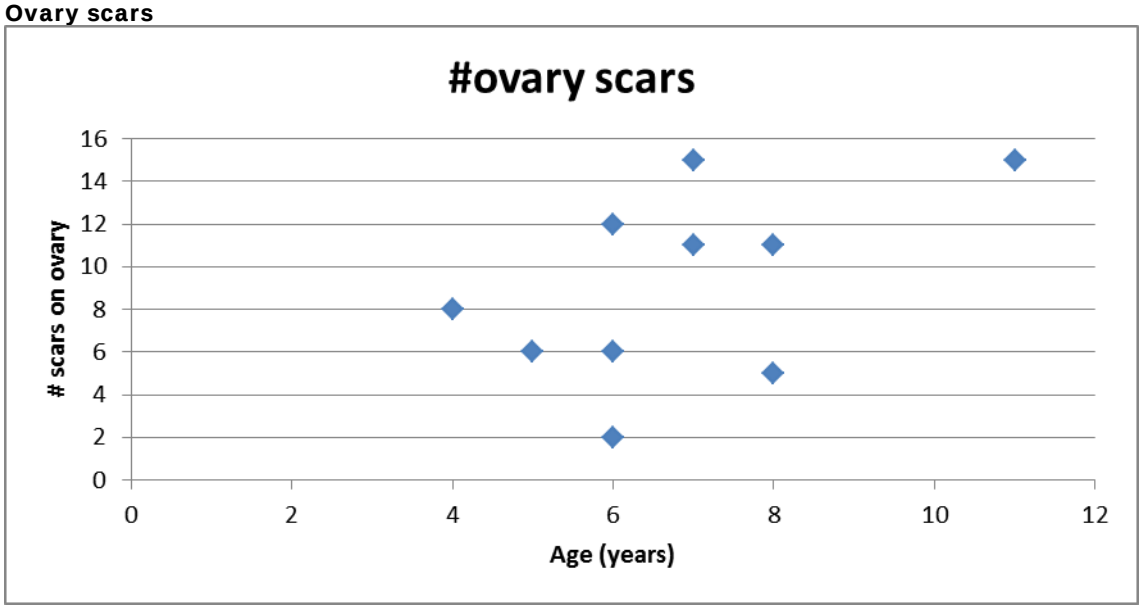

Figure A.8 *Number of ovary scars per age in female harbour porpoises, beached along the Dutch coast.*

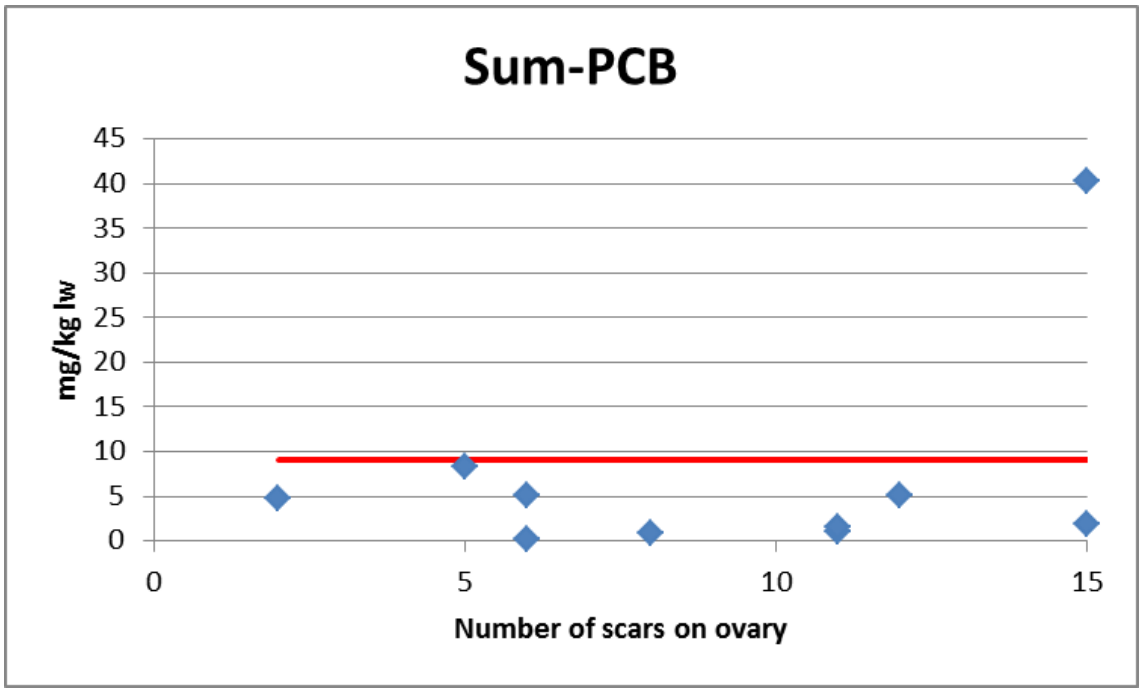

Figure A.9 *Sum-17PCB concentrations (in mg/kg lw) in blubber samples in relation to the number of ovarian scars of female harbour porpoises, beached along the Dutch coast (based on lipid weight). The safe level for Sum-PCBs is set at 9 mg/kg lipid weight (Jepson e.a., 2016). Results of samples of harbour porpoises with a NCC>4 were excluded.*

Comparison between age classes

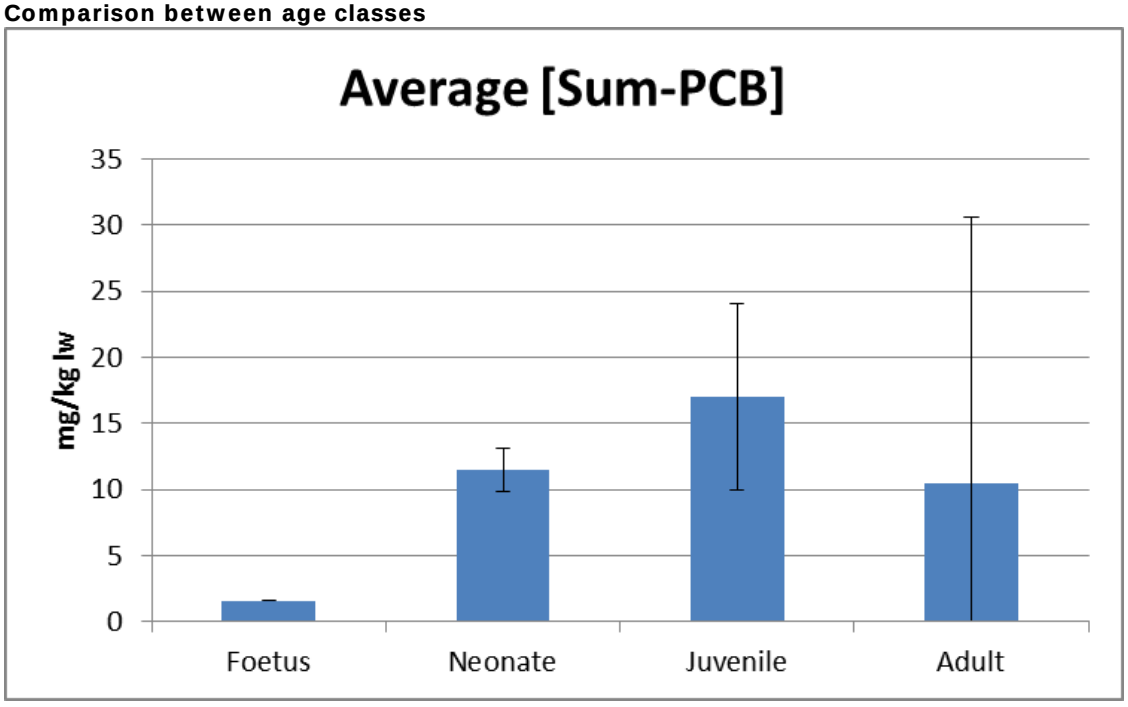

Figure A.10 *Sum-17PCB concentrations (in mg/kg lw) in blubber samples of all age classes of female harbour porpoises, beached along the Dutch coast (based on lipid weight). The safe level for Sum-PCBs is set at 9 mg/kg lipid weight (Jepson e.a., 2016). For statistical analysis, see Annex G.*

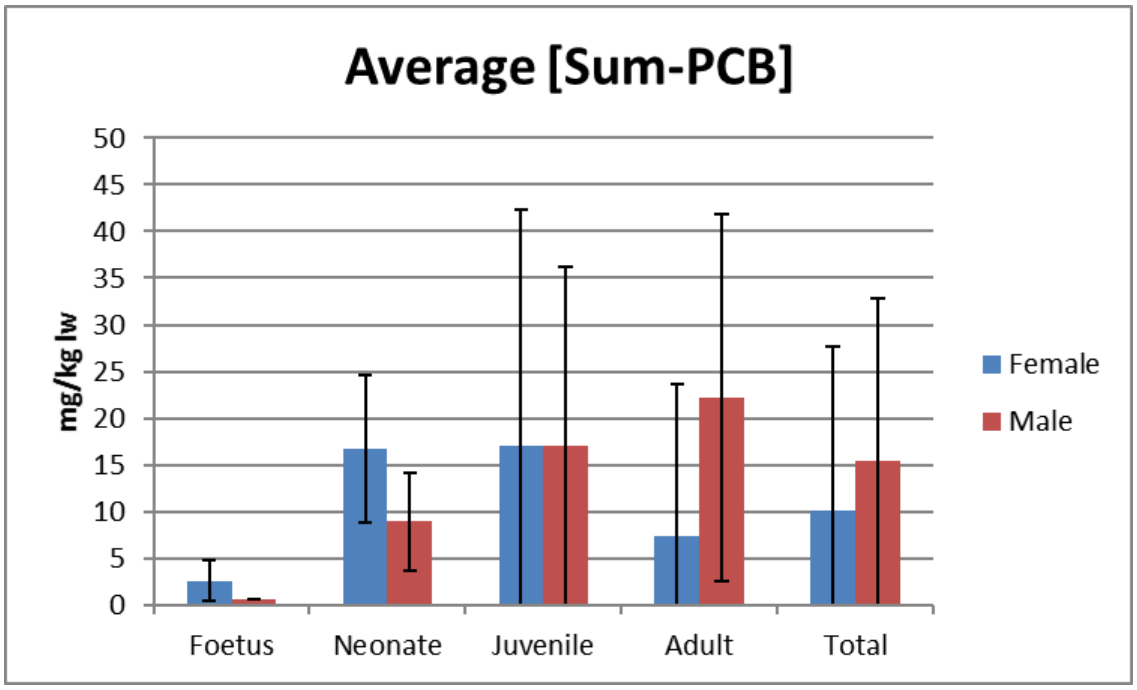

Figure A.11 *Sum-17PCB concentrations (in mg/kg lw) in blubber samples of all age classes of female and male harbour porpoises, beached along the Dutch coast (based on lipid weight). The safe level for Sum-PCBs is set at 9 mg/kg lipid weight (Jepson e.a., 2016). For statistical analysis, see Annex G.*

Annex B PBDE figures

This annex presents additional figures to the figures that are incorporated in the main text.

Nutritional status versus PBDE levels

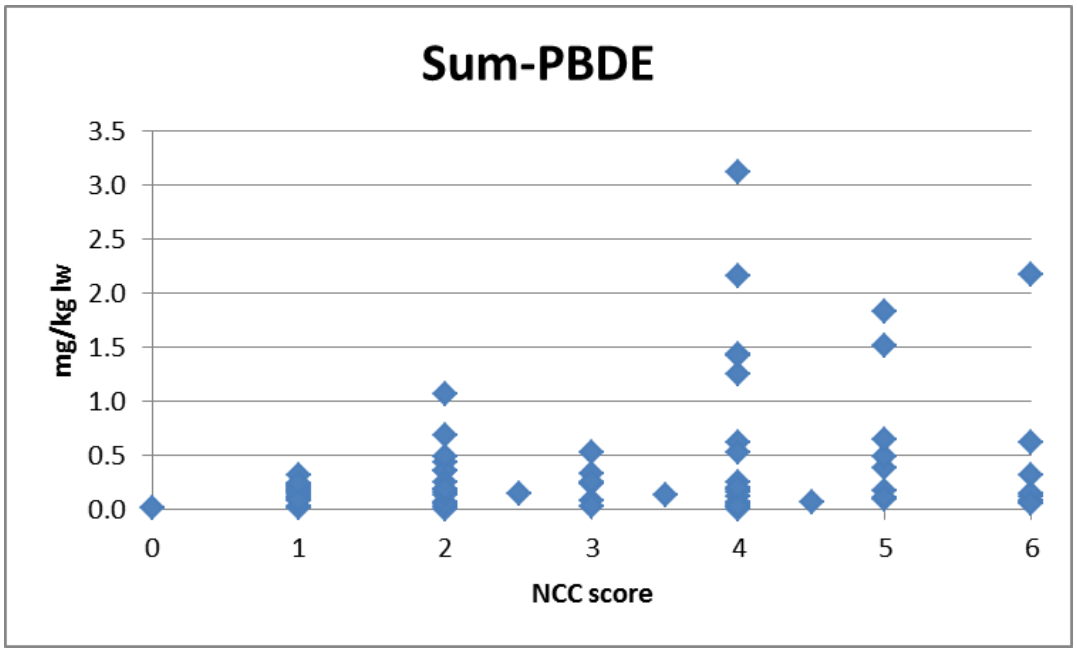

Figure B.1 *Sum-6PBDE concentrations (in mg/kg lw) in blubber samples in relation to NCC score of harbour porpoises, beached along the Dutch coast (based on lipid weight).*

Age versus PBDE levels

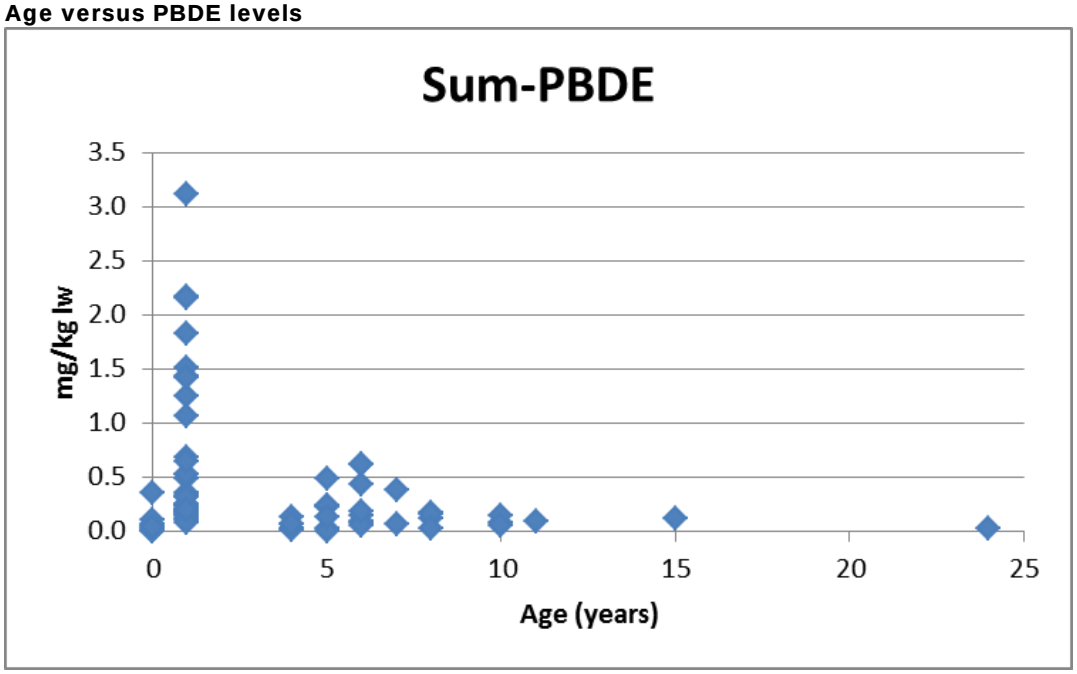

Figure B.2 *Sum-6PBDE concentrations (in mg/kg lw) in blubber samples in relation to age of harbour porpoises, beached along the Dutch coast (based on lipid weight).*

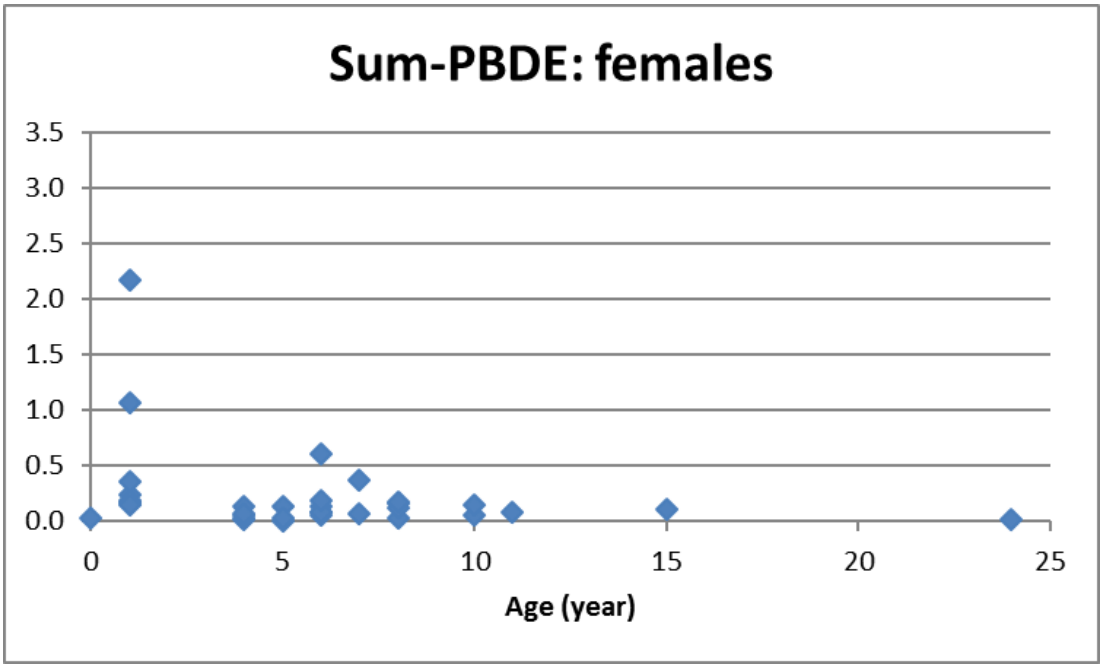

Figure B.3 *Sum-6PBDE concentrations (in mg/kg lw) in blubber samples in relation to age of female harbour porpoises, beached along the Dutch coast (based on lipid weight).*

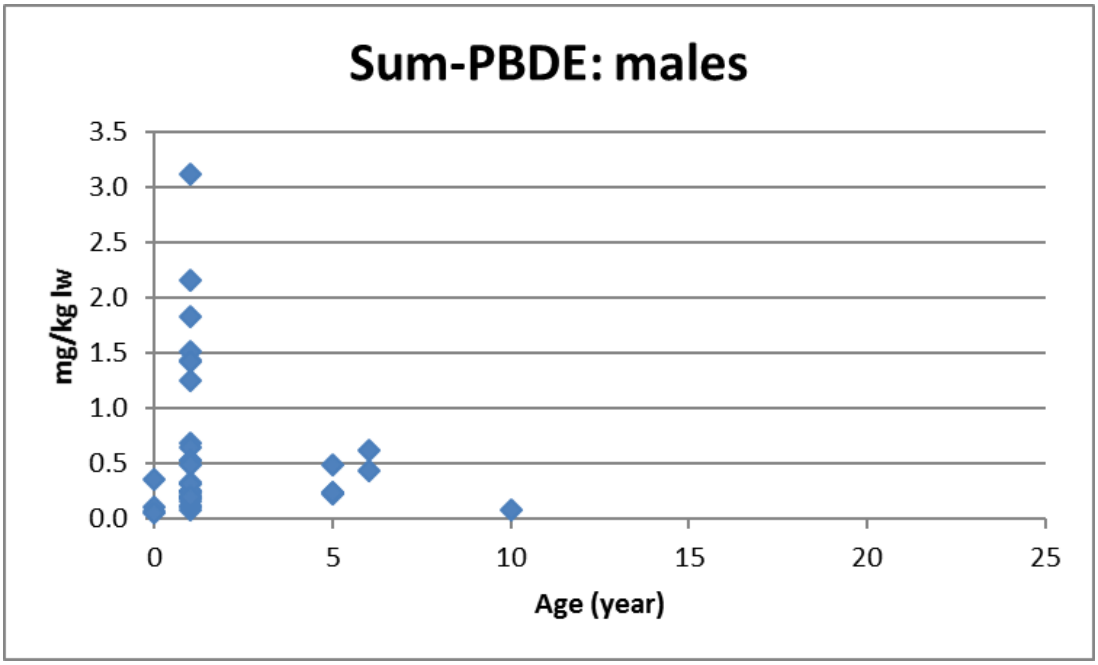

Figure B.4 *Sum-6PBDE concentrations (in mg/kg lw) in blubber samples in relation to age of male harbour porpoises, beached along the Dutch coast (based on lipid weight).*

Length versus PBDE levels

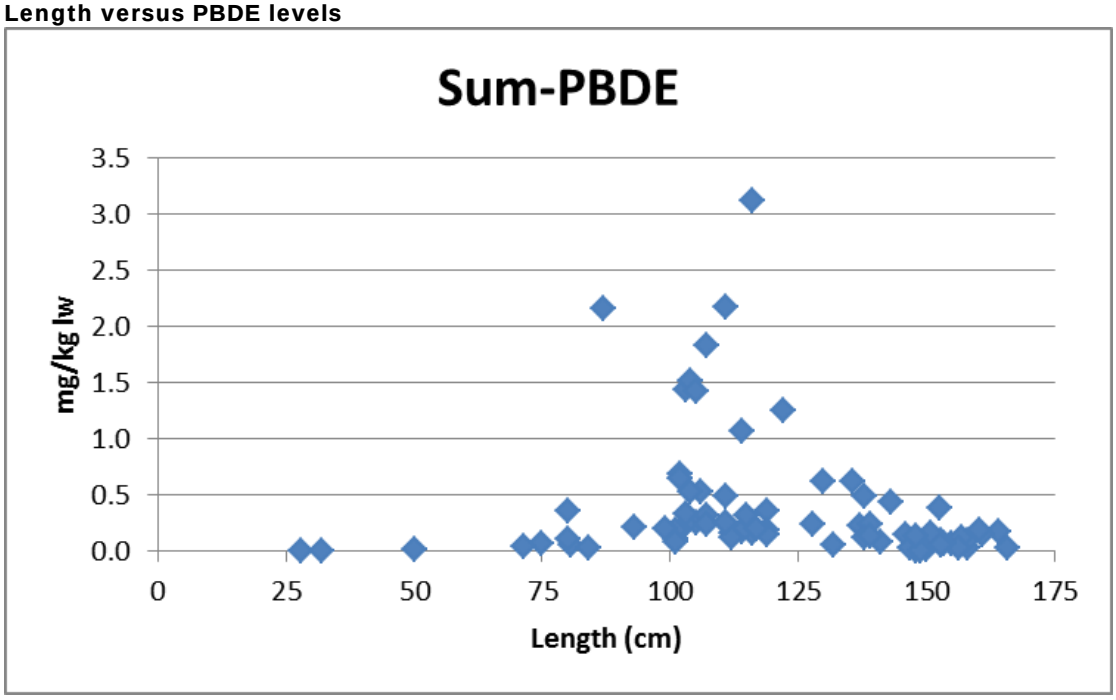

Figure B.5 *Sum-6PBDE concentrations (in mg/kg lw) in blubber samples in relation to body length of harbour porpoises, beached along the Dutch coast (based on lipid weight).*

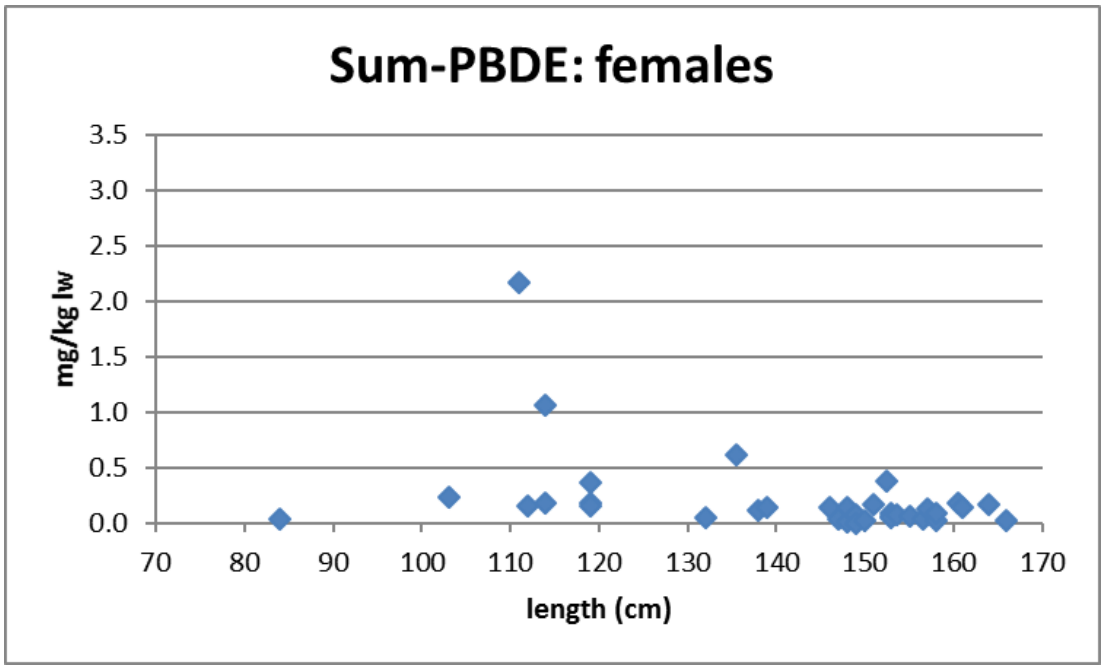

Figure B.6 *Sum-6PBDE concentrations (in mg/kg lw) in blubber samples in relation to body length of female harbour porpoises, beached along the Dutch coast (based on lipid weight).*

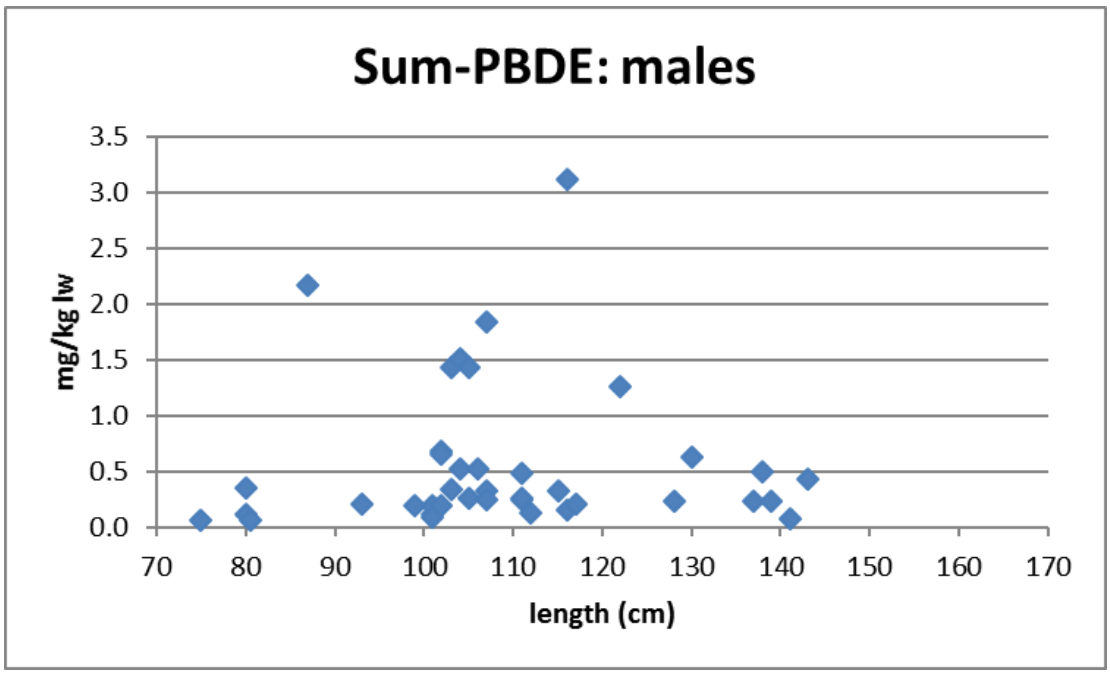

Figure B.7 *Sum-6PBDE concentrations (in mg/kg lw) in blubber samples in relation to body length of male harbour porpoises, beached along the Dutch coast (based on lipid weight).*

Number of ovary scars versus PBDE levels

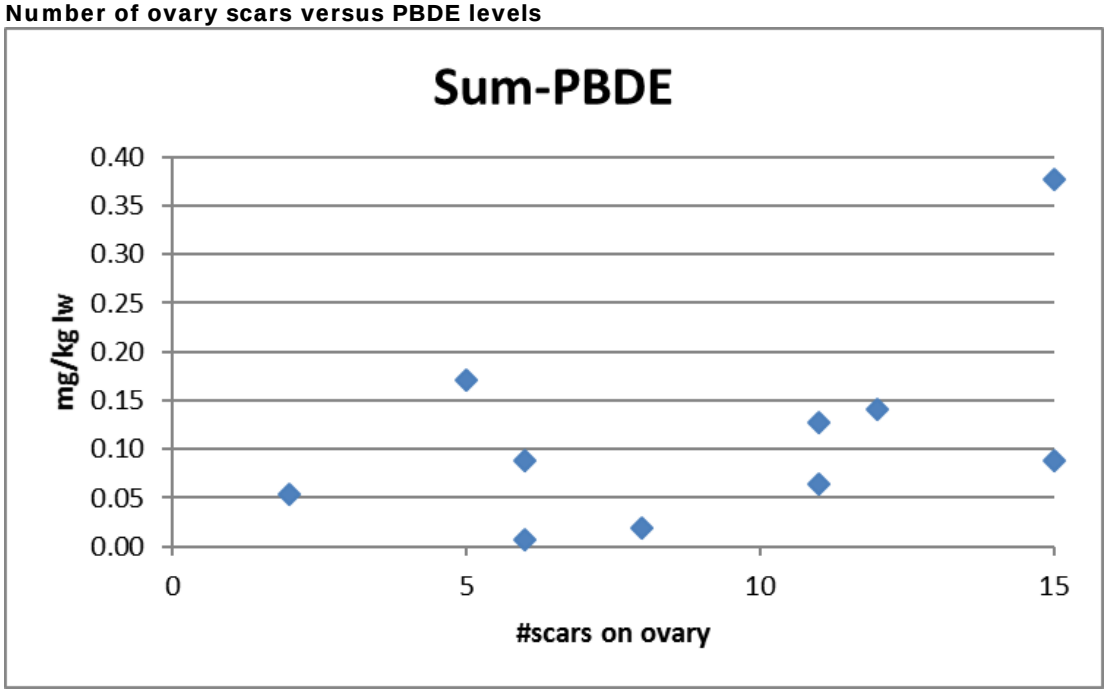

Figure B.8 *Sum-6PBDE concentrations (in mg/kg lw) in blubber samples in relation to the number of ovarian scars of female harbour porpoises, beached along the Dutch coast (based on lipid weight).*

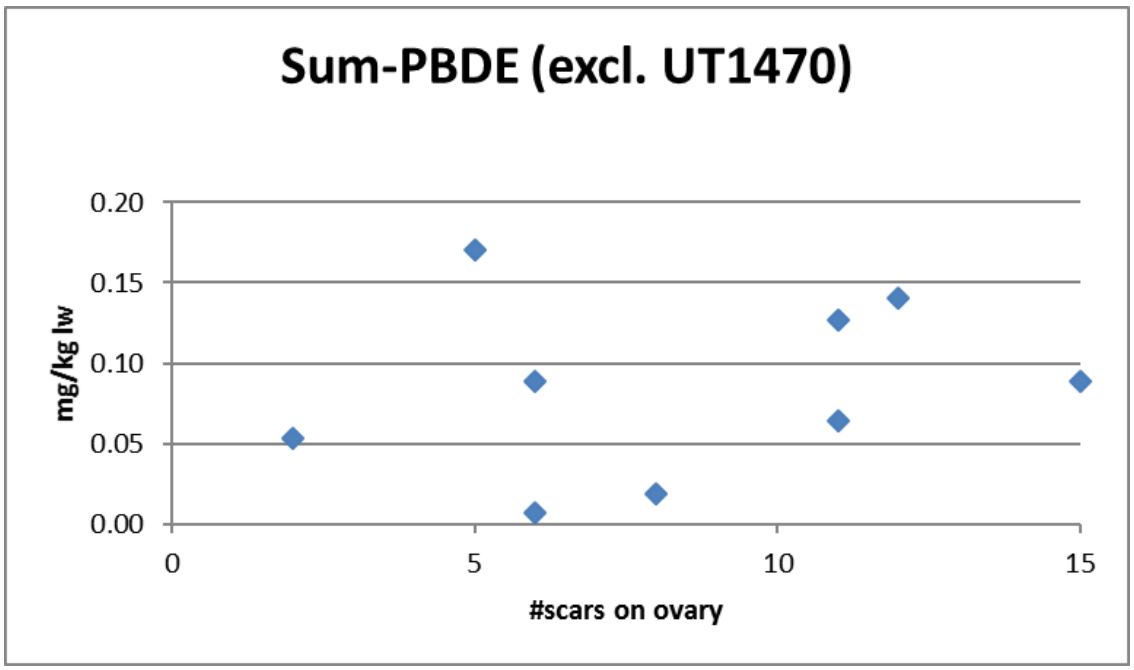

Figure B.9 *Sum-6PBDE concentrations (in mg/kg lw) in blubber samples in relation to the number of ovarian scars of female harbour porpoises, beached along the Dutch coast (based on lipid weight). Results of sample UT1470 of harbour porpoises was excluded to better view results of the other samples..*

Comparison between age classes

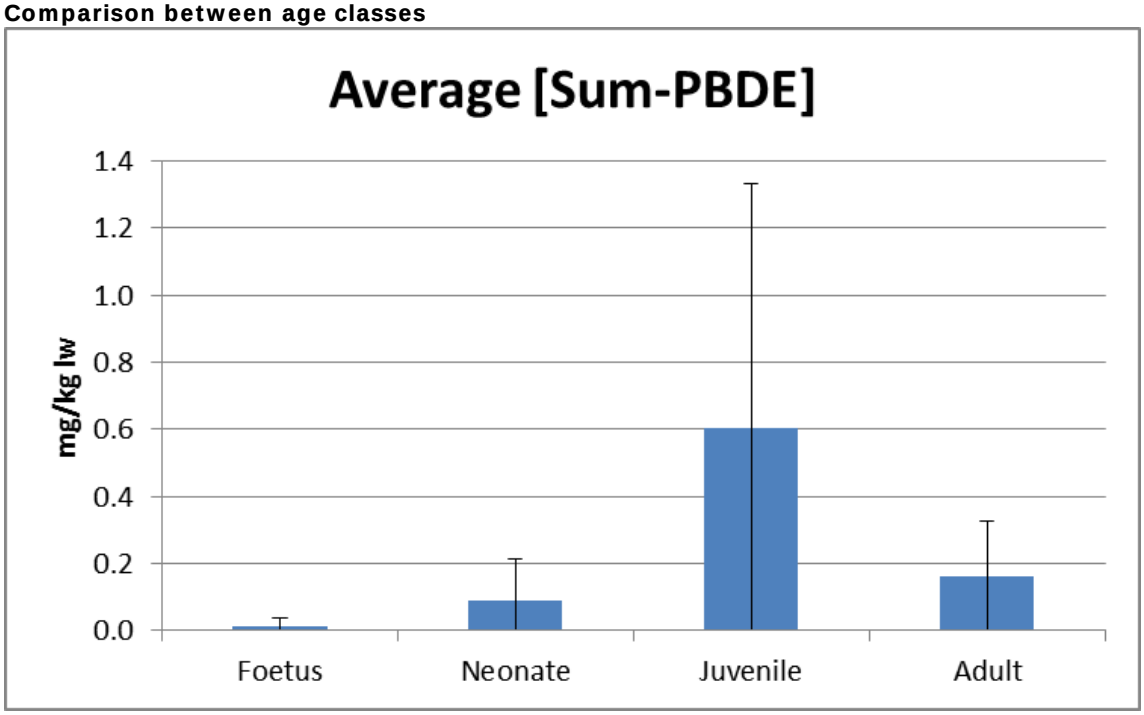

Figure B.10 *Sum-6PBDE concentrations (in mg/kg lw) in blubber samples of all age classes of harbour porpoises, beached along the Dutch coast (based on lipid weight).*

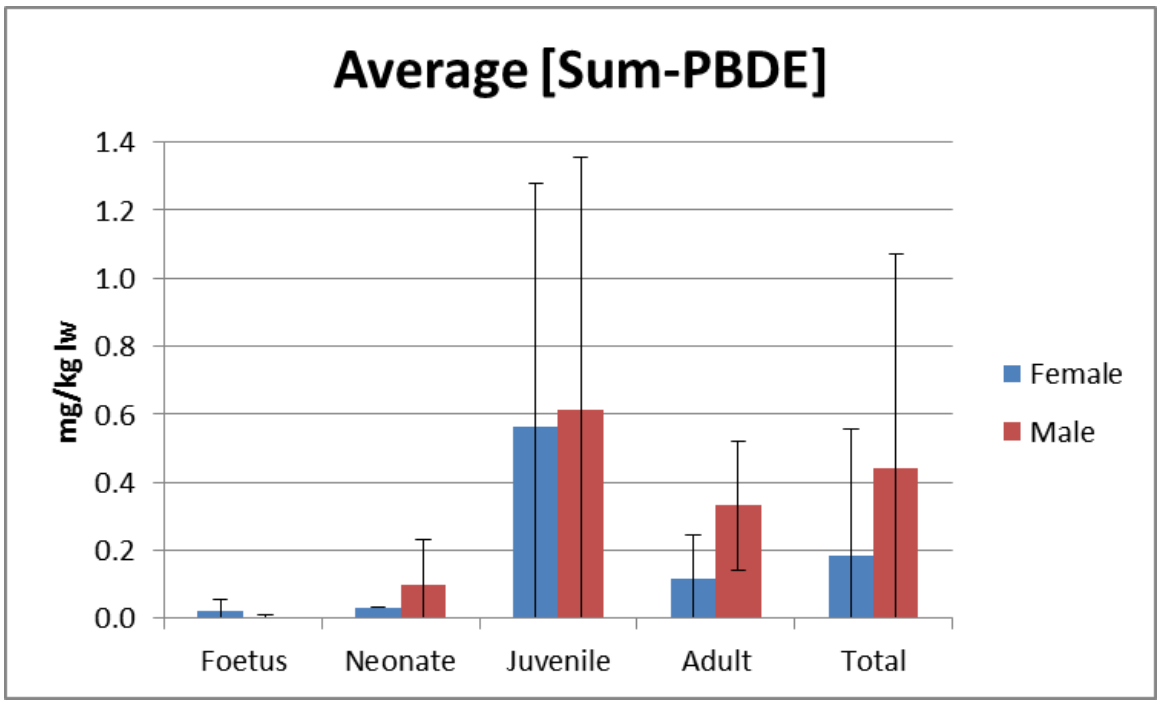

Figure B.11 *Sum-6PBDE concentrations (in mg/kg lw) in blubber samples of all age classes of harbour porpoises, beached along the Dutch coast (based on lipid weight).*

Annex C HCB figures

This annex presents additional figures to the figures that are incorporated in the main text.

Nutritional status versus HCB levels

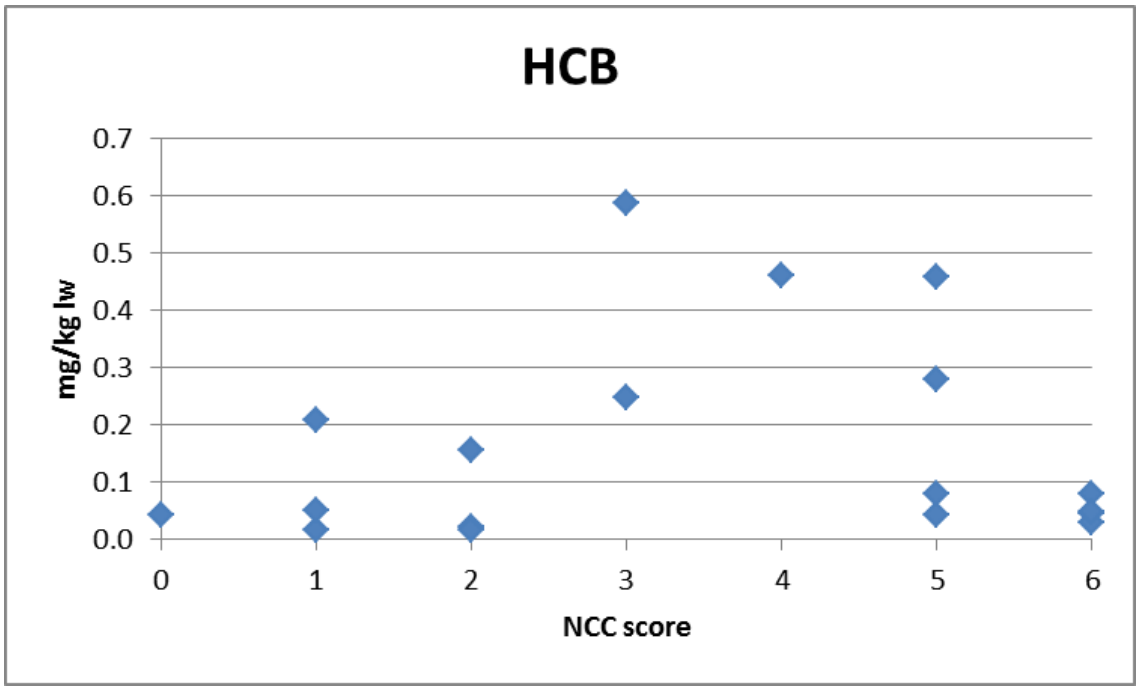

Figure C.1 *HCB concentrations (in mg/kg lw) in blubber samples in relation to NCC score of harbour porpoises, beached along the Dutch coast (based on lipid weight).*

Age versus HCB levels

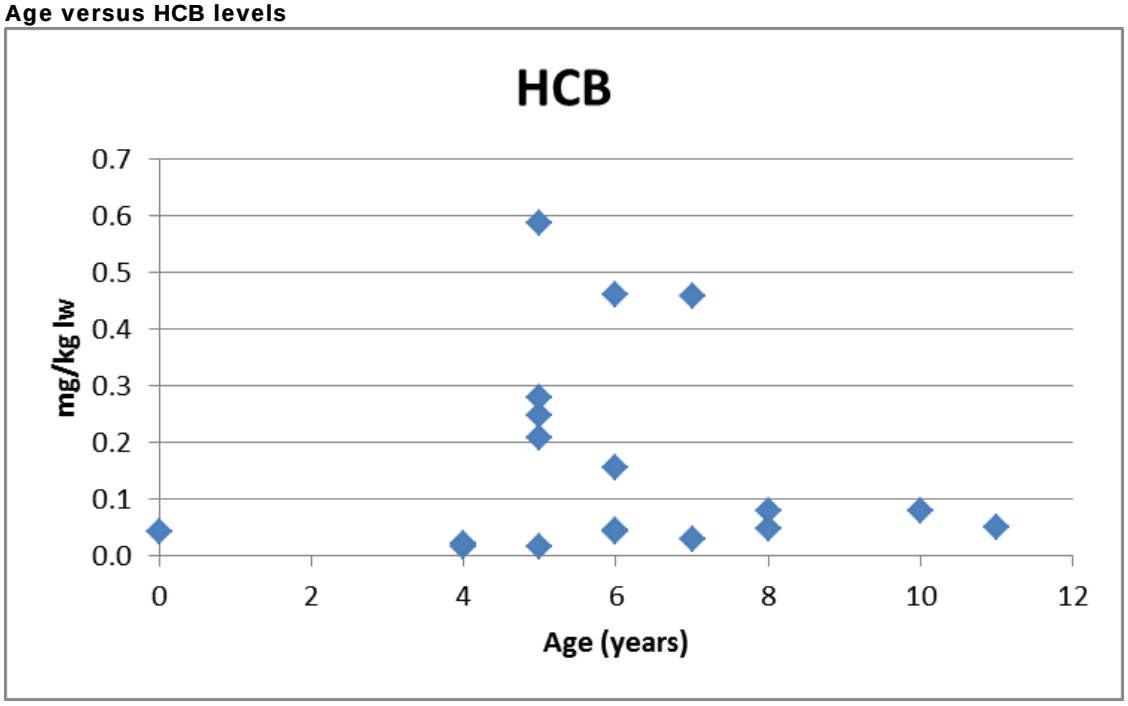

Figure C.2 *HCB concentrations (in mg/kg lw) in blubber samples in relation to age of harbour porpoises, beached along the Dutch coast (based on lipid weight).*

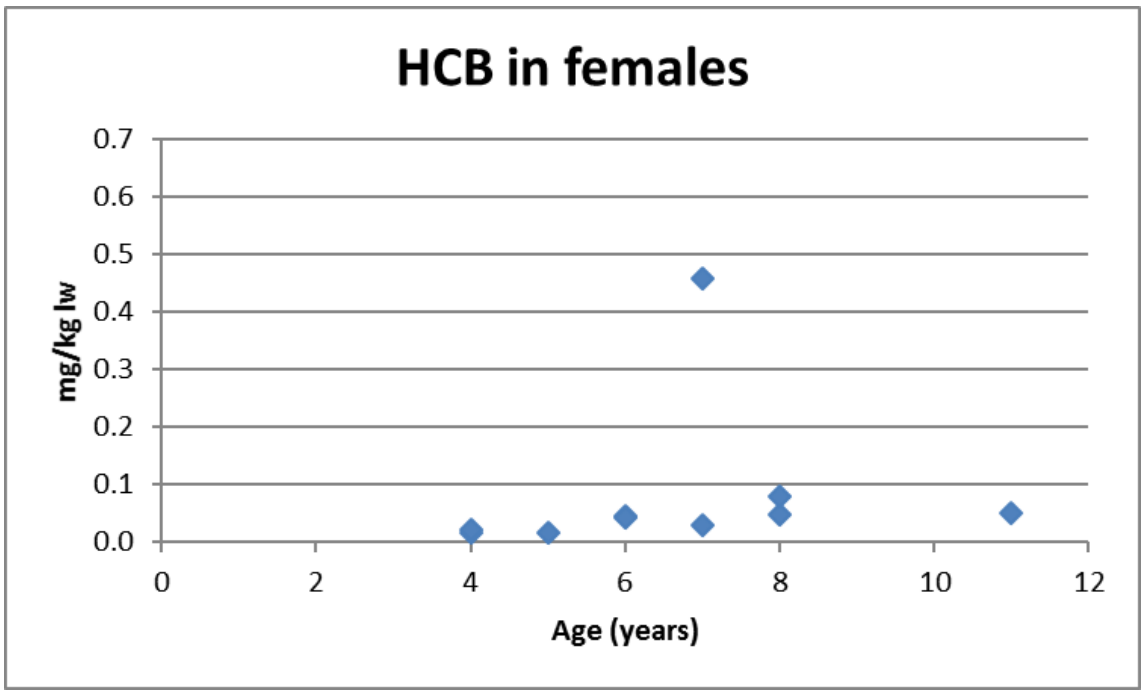

Figure C.3 *HCB concentrations (in mg/kg lw) in blubber samples in relation to age of female harbour porpoises, beached along the Dutch coast (based on lipid weight).*

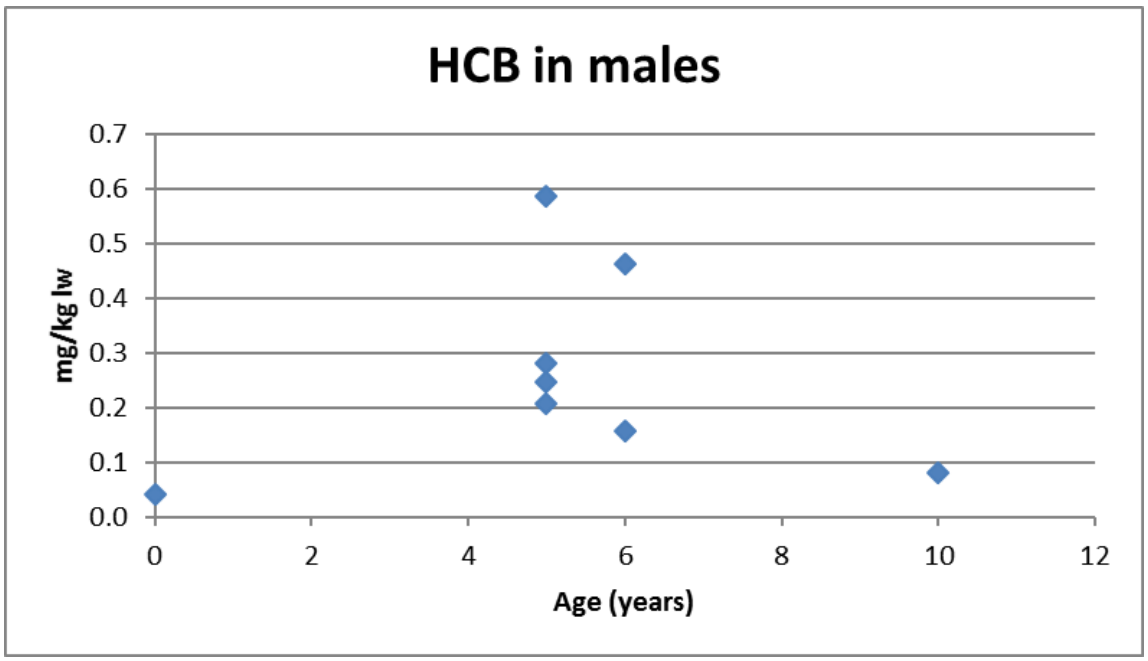

Figure C.4 *HCB concentrations (in mg/kg lw) in blubber samples in relation to age of male harbour porpoises, beached along the Dutch coast (based on lipid weight).*

Length versus HCB levels

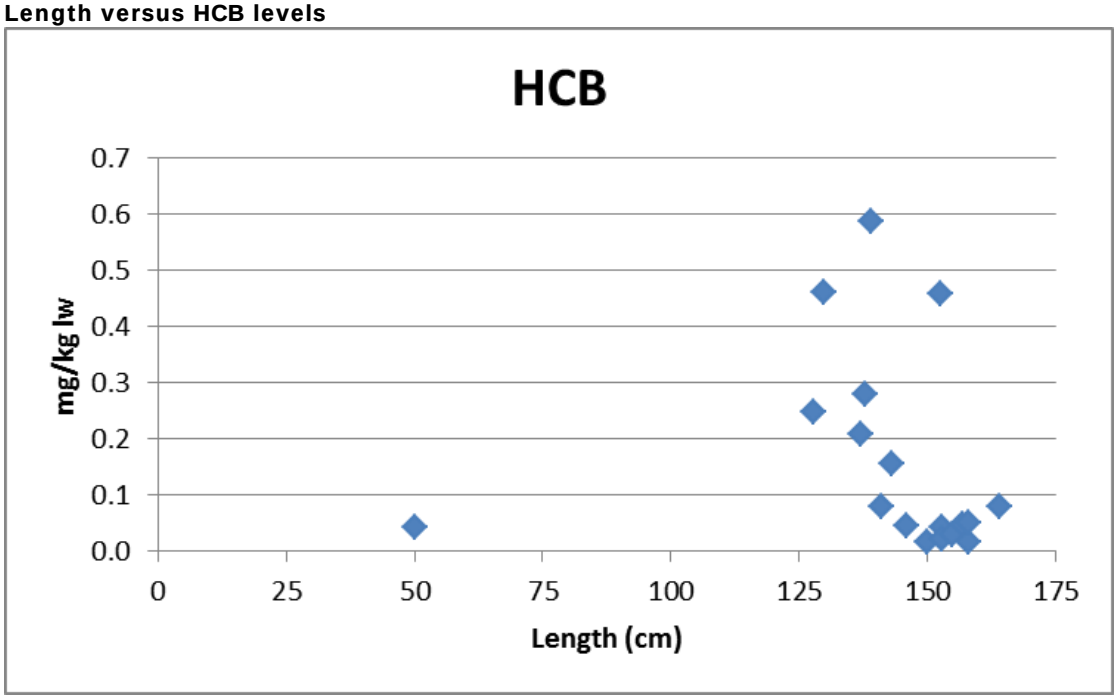

Figure C.5 *HCB concentrations (in mg/kg lw) in blubber samples in relation to body length of harbour porpoises, beached along the Dutch coast (based on lipid weight).*

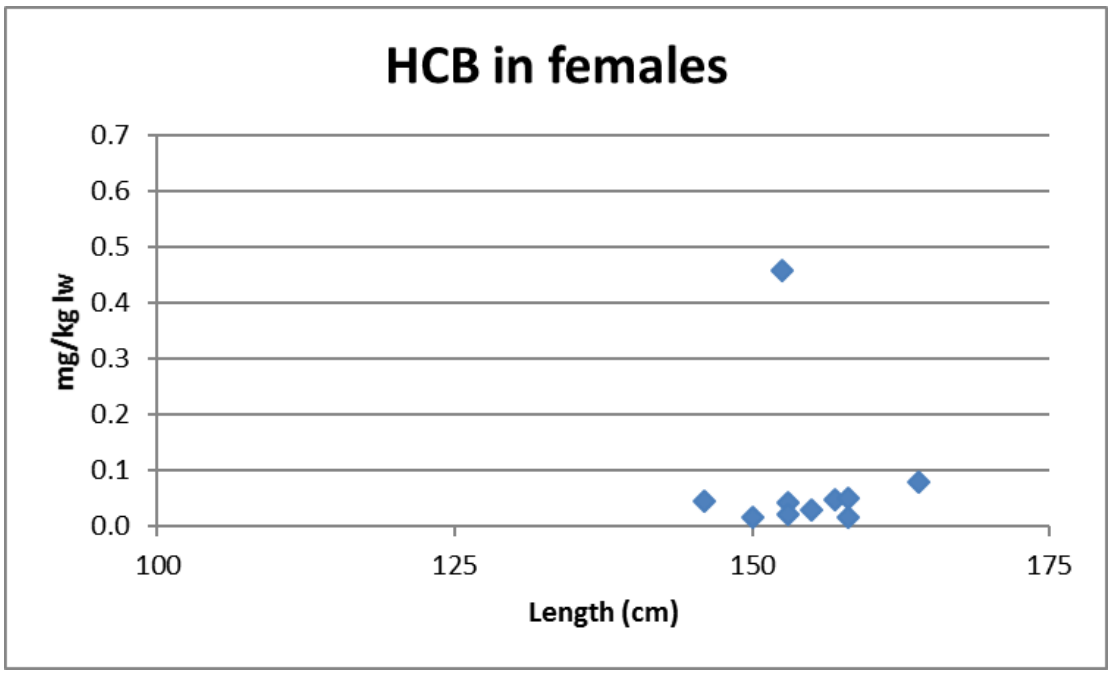

Figure C.6 *HCB concentrations (in mg/kg lw) in blubber samples in relation to body length of female harbour porpoises, beached along the Dutch coast (based on lipid weight). Results of samples of harbour porpoises with a NCC>4 were excluded.*

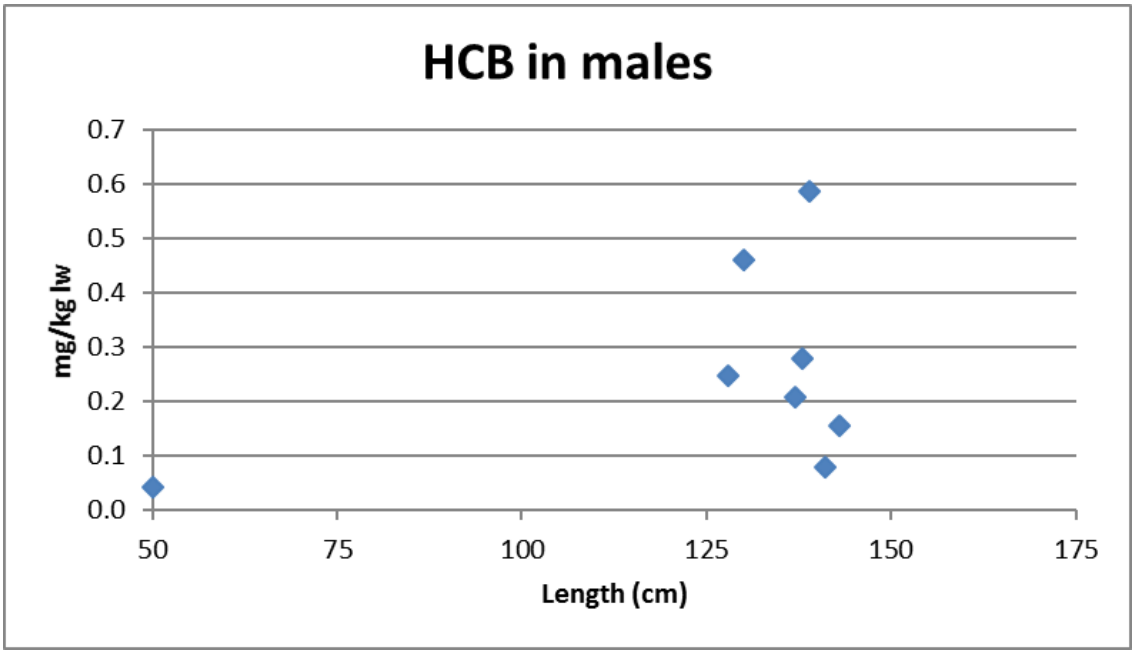

Figure C.7 *HCB concentrations (in mg/kg lw) in blubber samples in relation to body length of male harbour porpoises, beached along the Dutch coast (based on lipid weight).*

Number of ovary scars versus HCB levels

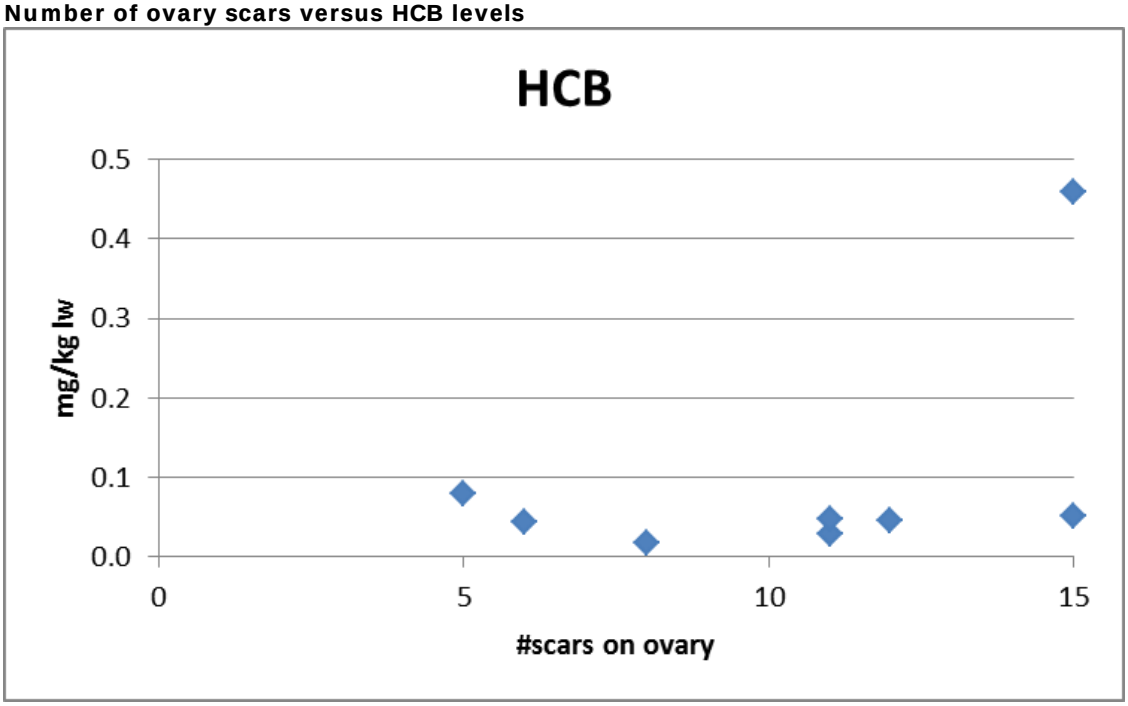

Figure C.8 *HCB concentrations (in mg/kg lw) in blubber samples in relation to the number of ovarian scars of female harbour porpoises, beached along the Dutch coast (based on lipid weight).*

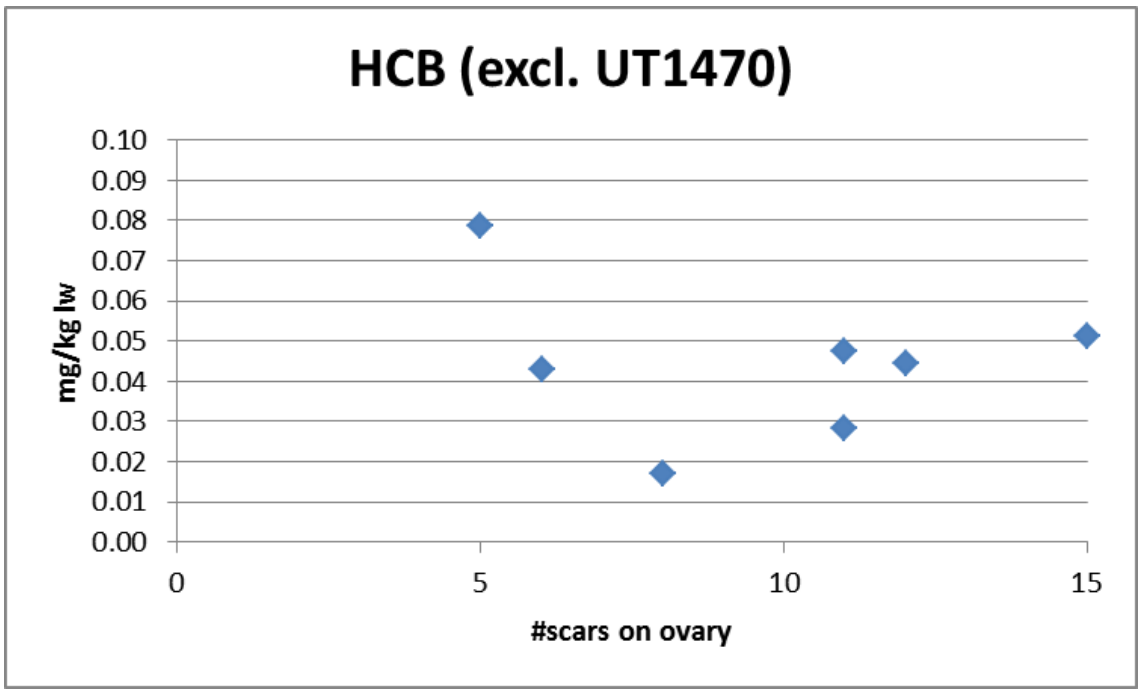

Figure C.9 *HCB concentrations (in mg/kg lw) in blubber samples in relation to the number of ovarian scars of female harbour porpoises, beached along the Dutch coast (based on lipid weight). Results of UT1470 were omitted from this figure to get a better view on the other results.*

Annex D Dioxin-like PCB figures

This annex presents additional figures to the figures that are incorporated in the main text.

Nutritional status versus dioxin-like PCB levels

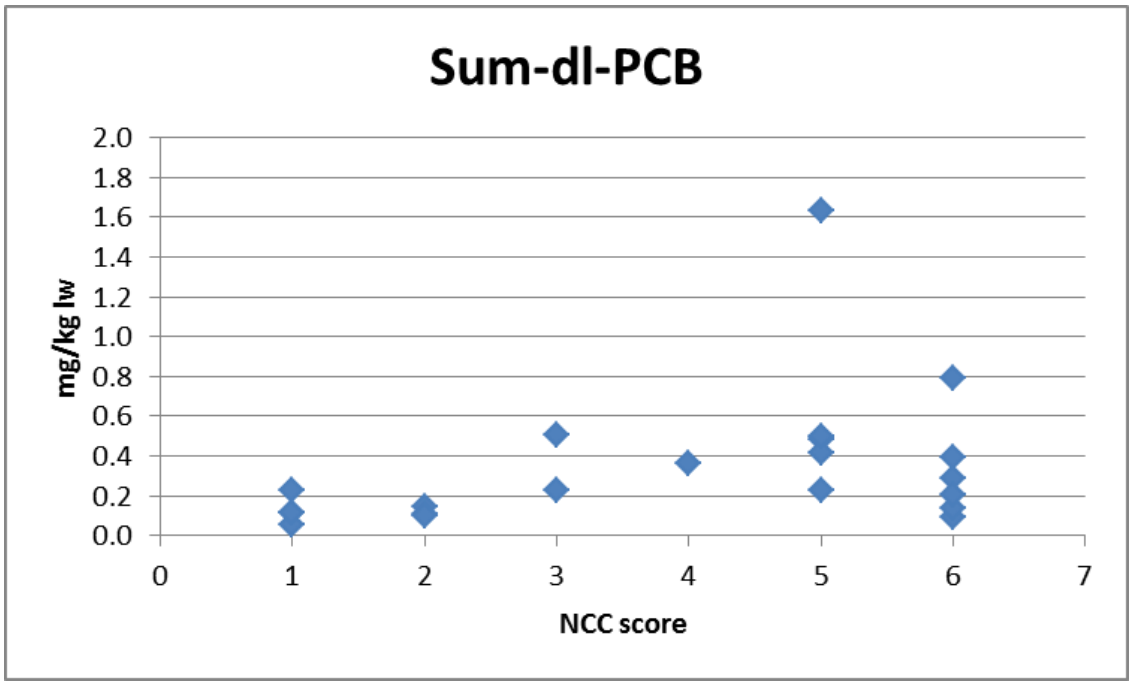

Figure D.1 *Dioxin-like PCB concentrations (in mg/kg lw) in blubber samples in relation to NCC score of harbour porpoises, beached along the Dutch coast (based on lipid weight).*

Length versus dioxin-like PCB levels

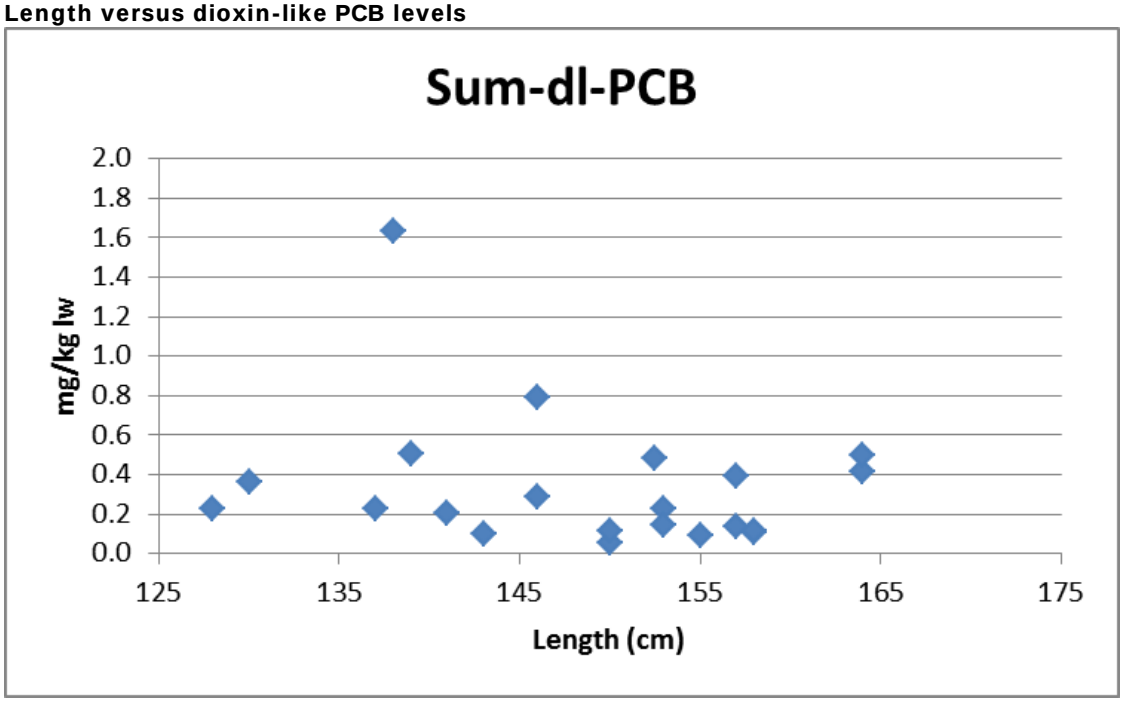

Figure D.2 *Dioxin-like PCB concentrations (in mg/kg lw) in blubber samples in relation to body length of harbour porpoises, beached along the Dutch coast (based on lipid weight).*

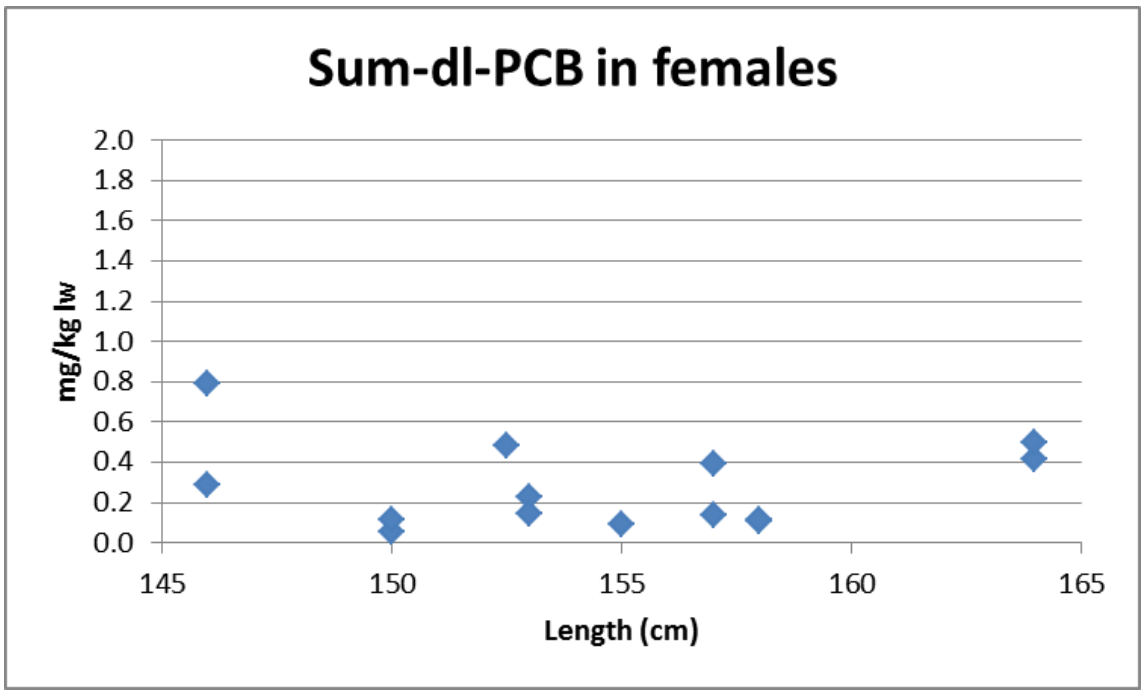

Figure D.3 *Dioxin-like PCB concentrations (in mg/kg lw) in blubber samples in relation to body length of female harbour porpoises, beached along the Dutch coast (based on lipid weight).*

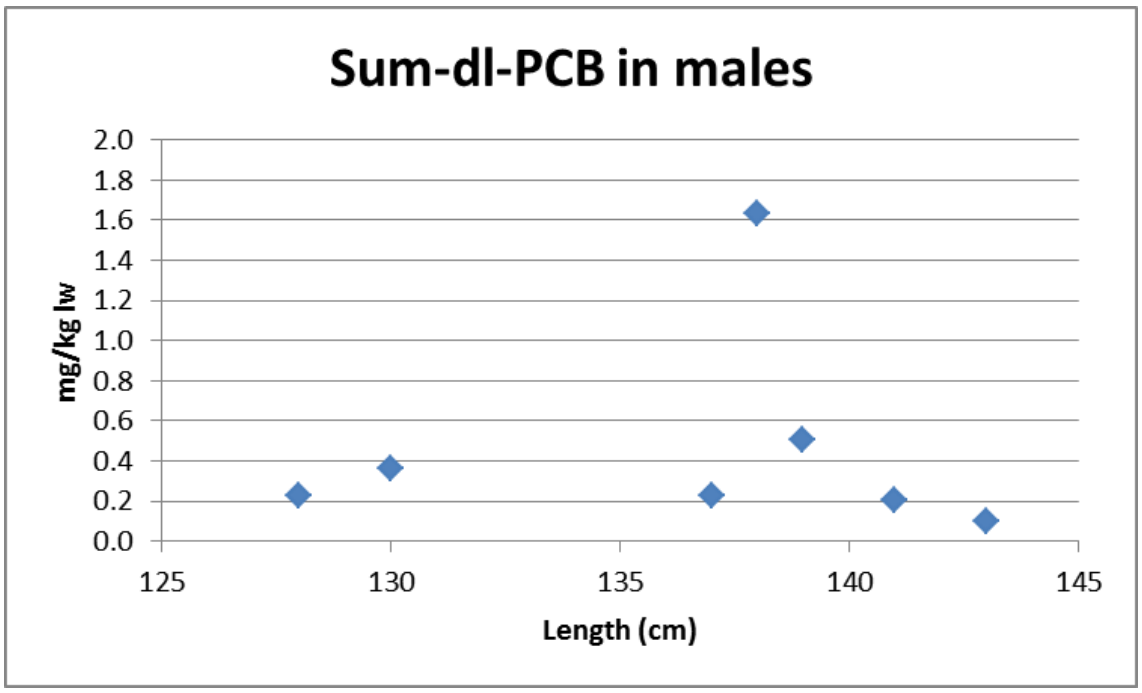

Figure D.4 *Dioxin-like PCB concentrations (in mg/kg lw) in blubber samples in relation to body length of male harbour porpoises, beached along the Dutch coast (based on lipid weight).*

Number of ovary scars versus dioxin-like PCB levels

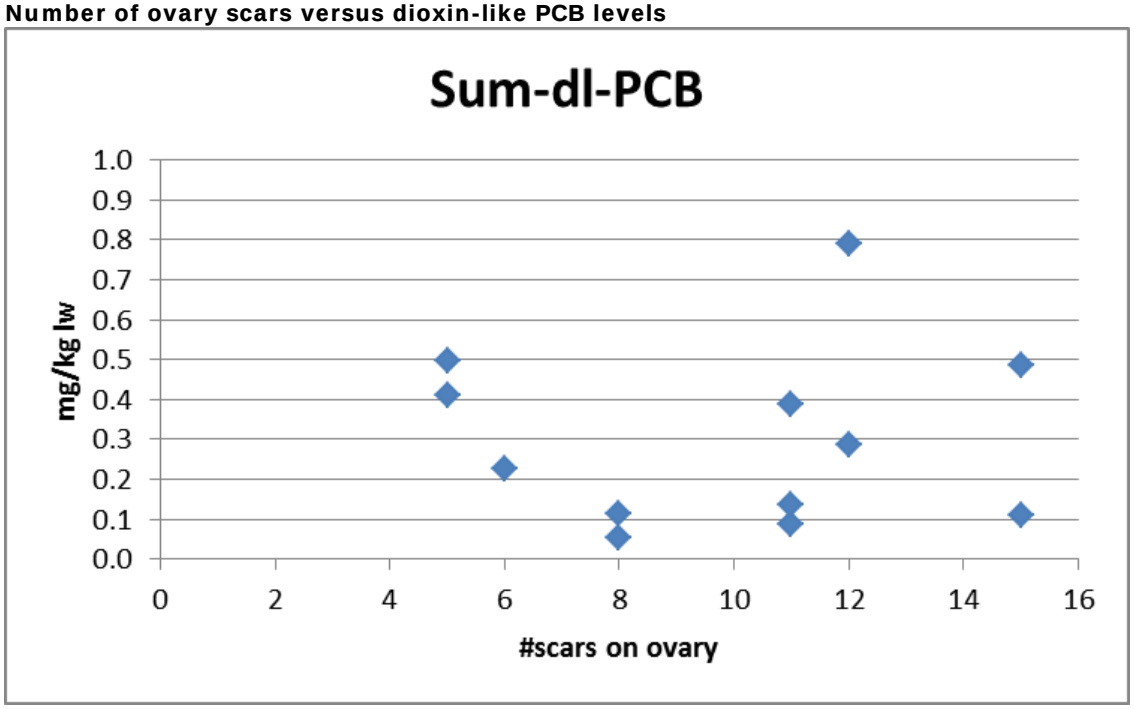

Figure D.5 *Dioxin-like PCB concentrations (in mg/kg lw) in blubber samples in relation to the number of ovarian scars of female harbour porpoises, beached along the Dutch coast (based on lipid weight). No deviating results were found for UT1470, so no separate figure is needed excluding UT1470.*

Comparison of dioxin-like PCB levels between sexes

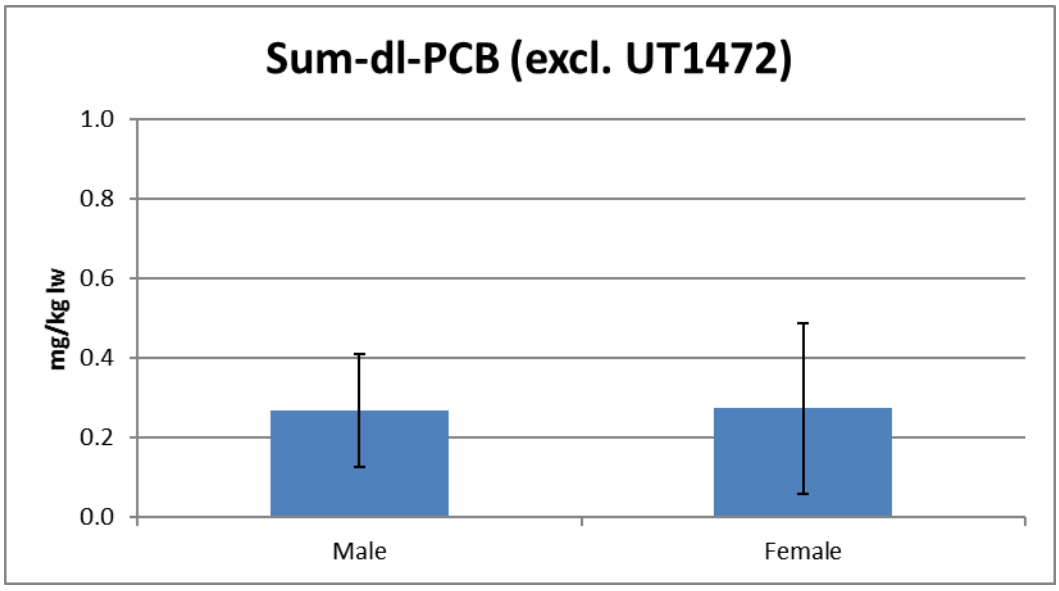

Figure D.6 *Dioxin-like PCB concentrations (in mg/kg lw) in blubber samples by sex of harbour porpoises, beached along the Dutch coast (based on lipid weight). The outlier of 1.64 mg/kg lw of UT1472 (1 5-year old adult male with an NCC of 5) was removed for this comparison. The figure including this outlier is presented in the main report.*

Annex E PFC figures

This annex presents additional figures to the figures that are incorporated in the main text.

Nutritional status versus PFC levels

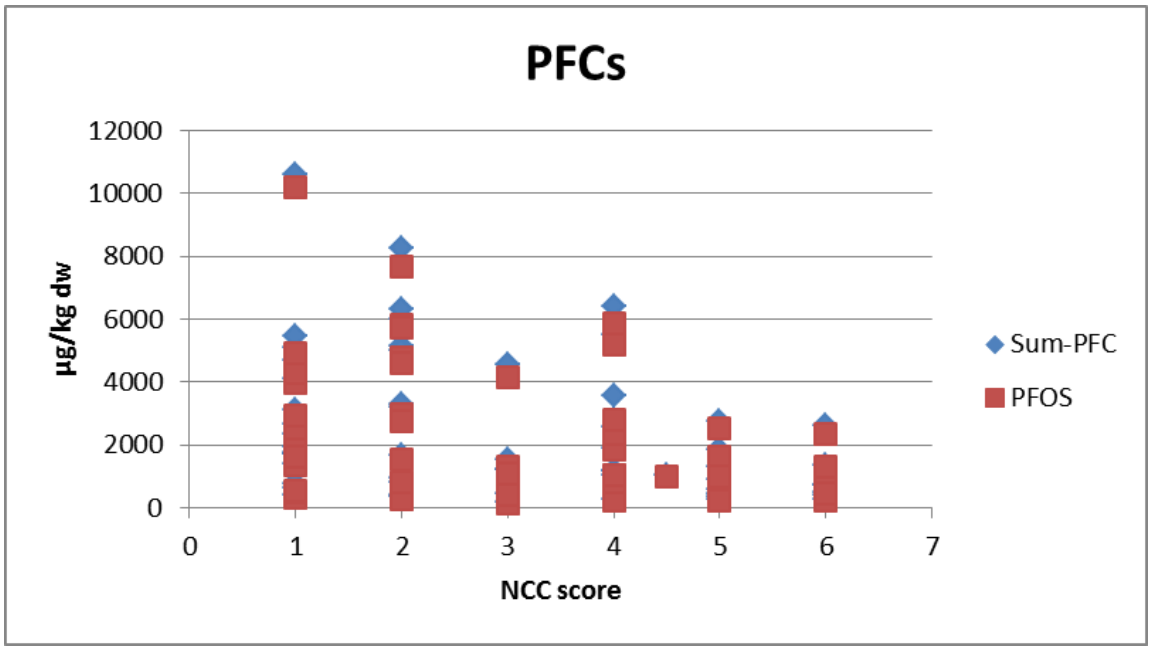

Figure E.1 *PFC concentrations (in µg/kg dw) in liver samples in relation to NCC score of harbour porpoises, beached along the Dutch coast (based on dry weight).*

Age versus PFC levels

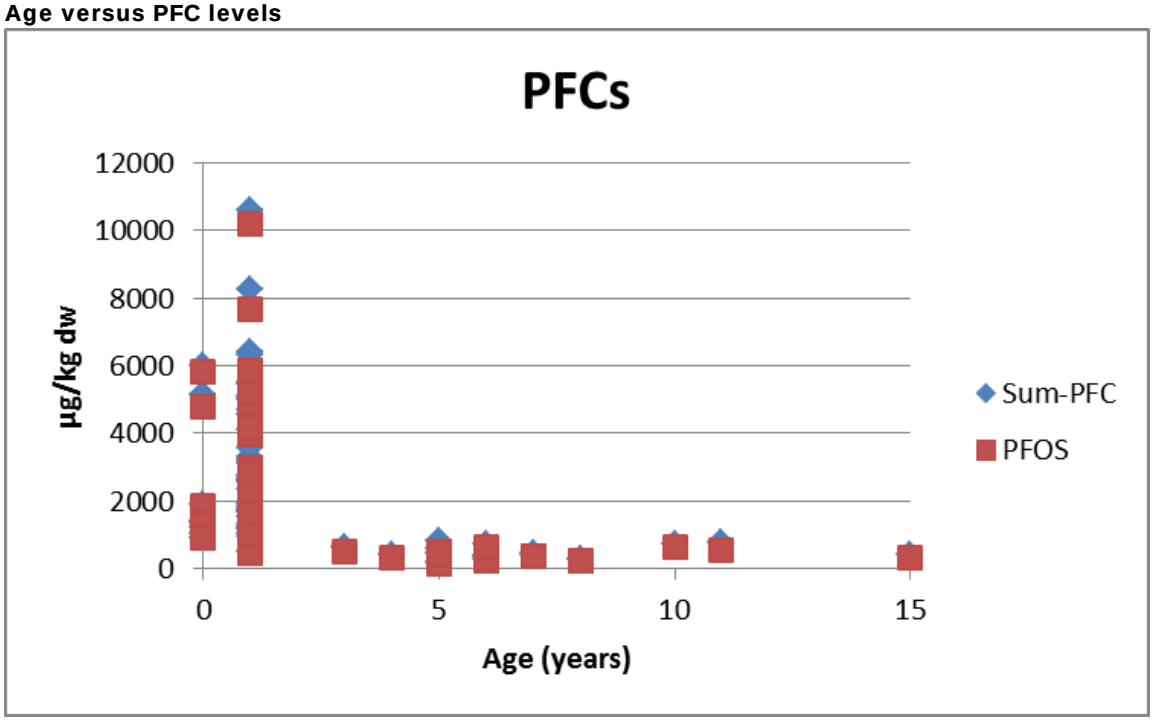

Figure E.2 *PFC concentrations (in µg/kg dw) in liver samples in relation to age of harbour porpoises, beached along the Dutch coast (based on dry weight).*

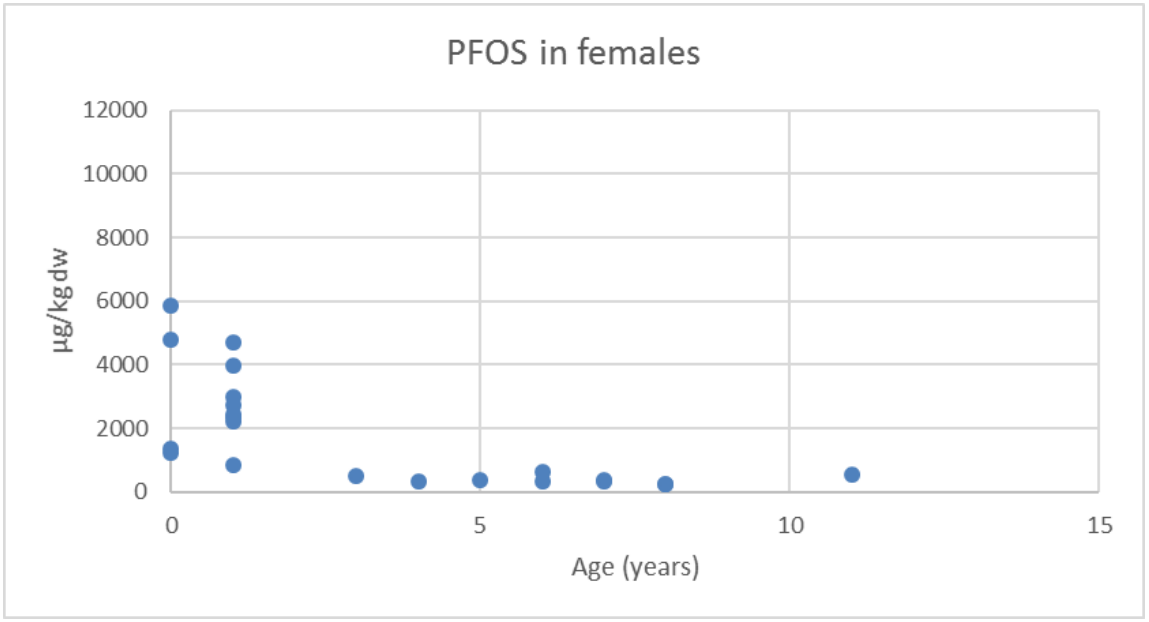

Figure E.3 *PFOS concentrations (in µg/kg dw) in liver samples in relation to age of female harbour porpoises, beached along the Dutch coast (based on dry weight). As can be seen in figure E1 and E.2 PFC concentrations are mainly based on PFOS concentrations. Therefore only PFOS data are shown here.*

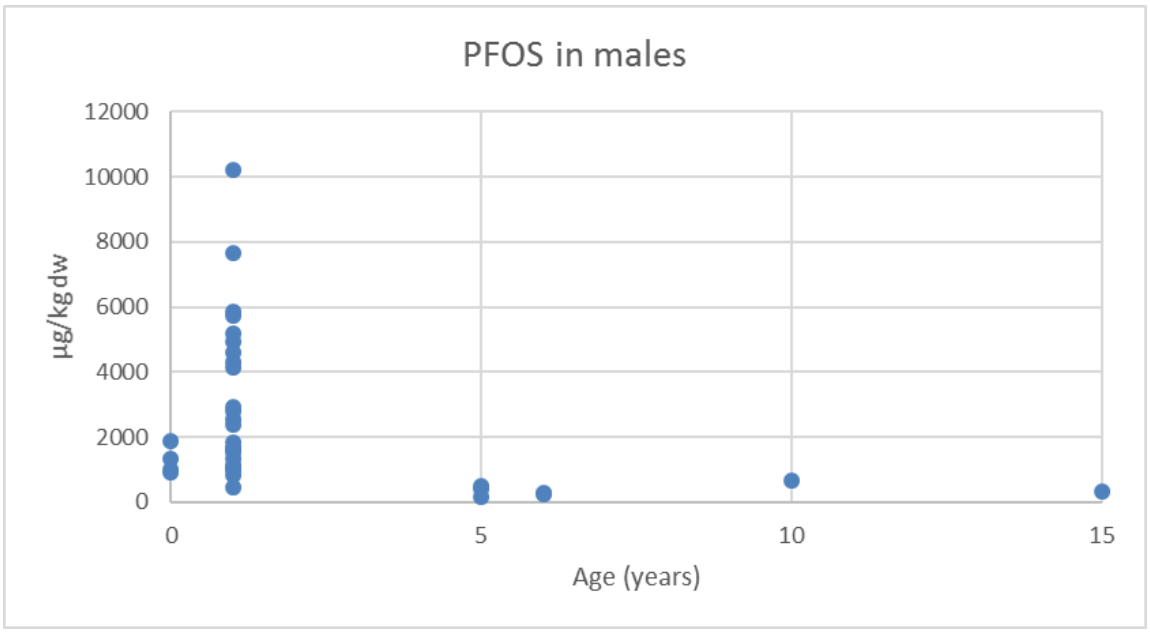

Figure E.4 *PFOS concentrations (in µg/kg dw) in liver samples in relation to age??? of male harbour porpoises, beached along the Dutch coast (based on dry weight). As can be seen in figure E1 and E.2 PFC concentrations are mainly based on PFOS concentrations. Therefore only PFOS data are shown here.*

Length versus PFOS levels

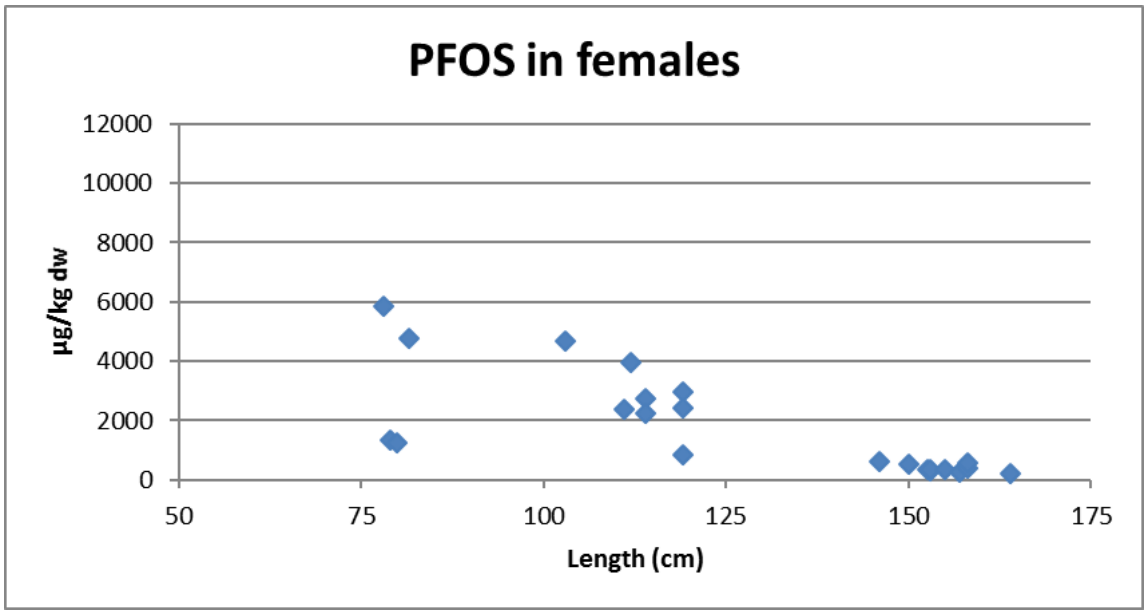

Figure E.5 *PFOS concentrations (in µg/kg dw) in liver samples in relation to body length of female harbour porpoises, beached along the Dutch coast (based on dry weight).*

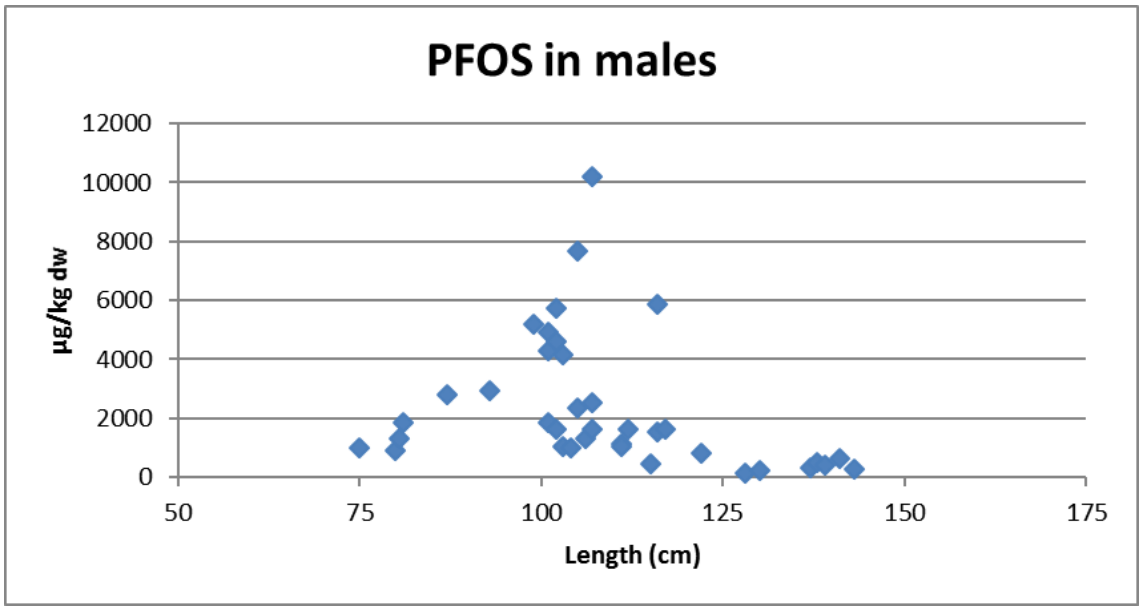

Figure E.6 *PFOS concentrations (in µg/kg dw) in liver samples in relation to body length of male harbour porpoises, beached along the Dutch coast (based on dry weight).*

Number of ovary scars versus PFC levels

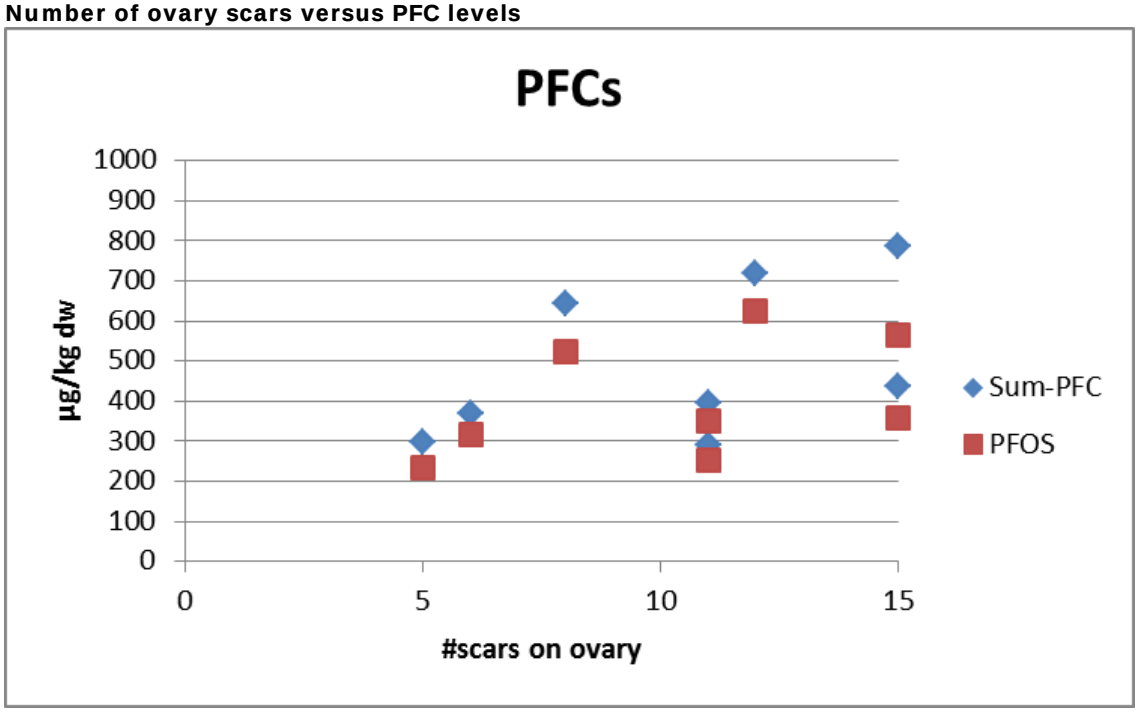

Figure E.7 *PFC concentrations (in µg/kg dw) in liver samples in relation to the number of ovarian scars of female harbour porpoises, beached along the Dutch coast (based on dry weight).*

Comparison between age classes

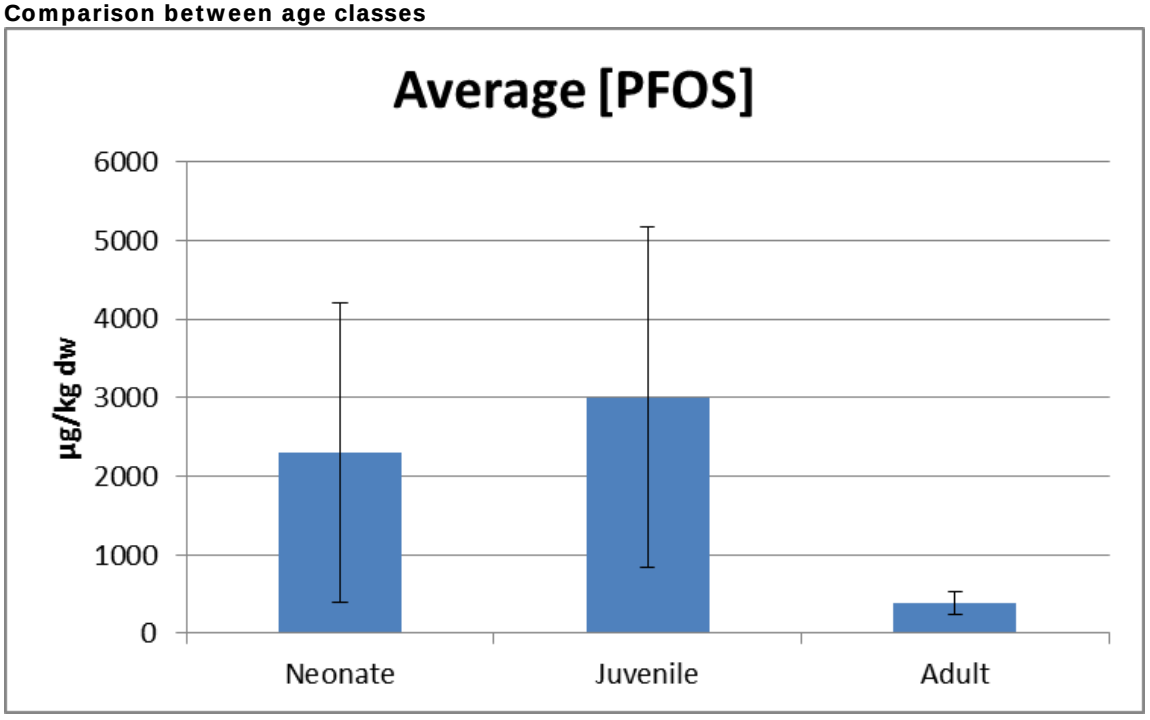

Figure E.8 *Average PFOS concentrations (in µg/kg dw) in liver samples in different age classes of harbour porpoises, beached along the Dutch coast (based on dry weight).*

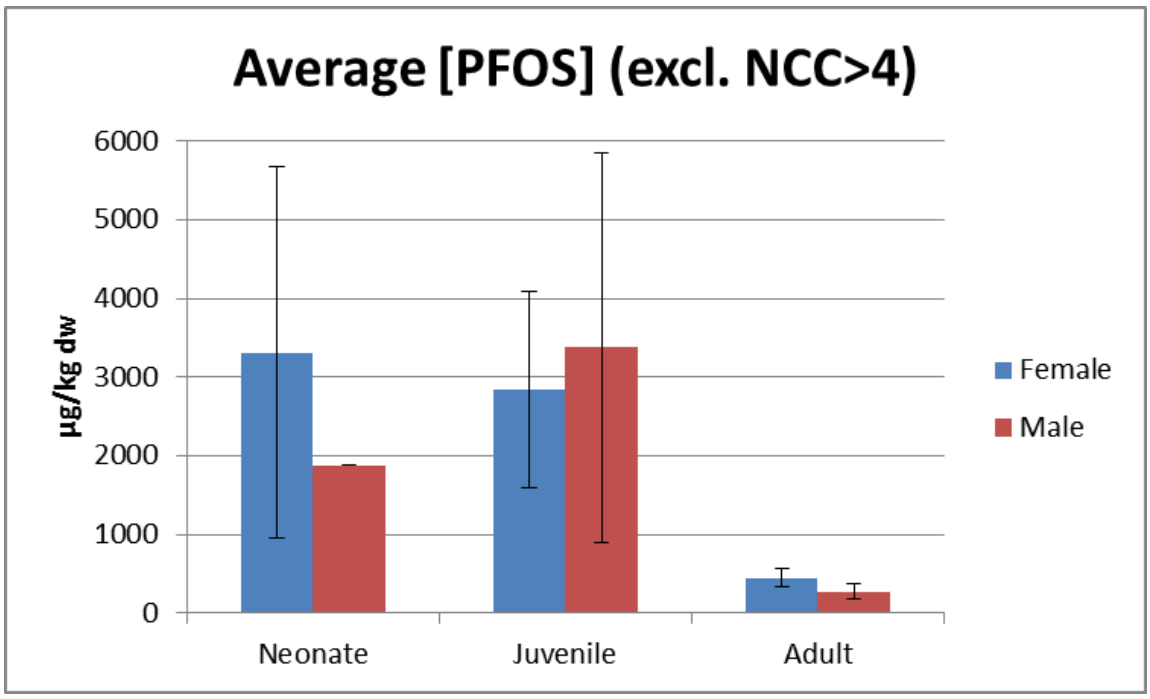

Figure E.9 *Average PFOS concentrations (in µg/kg dw) in liver samples in different age classes of female and male harbour porpoises, beached along the Dutch coast (based on dry weight). Results of samples of harbour porpoises with a NCC>4 were excluded. The overview of all data is presented in the main text.*

Annex F Overview of analysed congeners

PCB congeners analysed in this study

Sum-17PCB consists of:

- CB49
- CB52
- CB101
- CB105
- CB118
- CB128
- CB138
- CB149
- CB151
- CB153
- CB156
- CB170
- CB187
- CB180
- CB194
- CB202

Sum-7PCB consists of:

- CB52
- CB101
- CB118
- CB138
- CB153
- CB180

PBDE congeners analysed in this study

Sum-6PBDE consists of:

- BDE28
- BDE47
- BDE99
- BDE100
- BDE153
- BDE154

Annex G Statistical treatment PCBs and PBDEs

PCBs – all samples

Significant interaction term between age and sex ($p<0.05$).

Significant differences (Tukey HSD post-hoc comparison):

- M:A-F:A 0.0012802
- F:J-F:A 0.0262112
- M:J-F:A 0.0000086
- F:N-F:A 0.0214466
- M:F-M:A 0.0022369
- F:J-M:F 0.0128831
- M:J-M:F 0.0027368
- F:N-M:F 0.0073367
- M:N-M:F 0.0383806

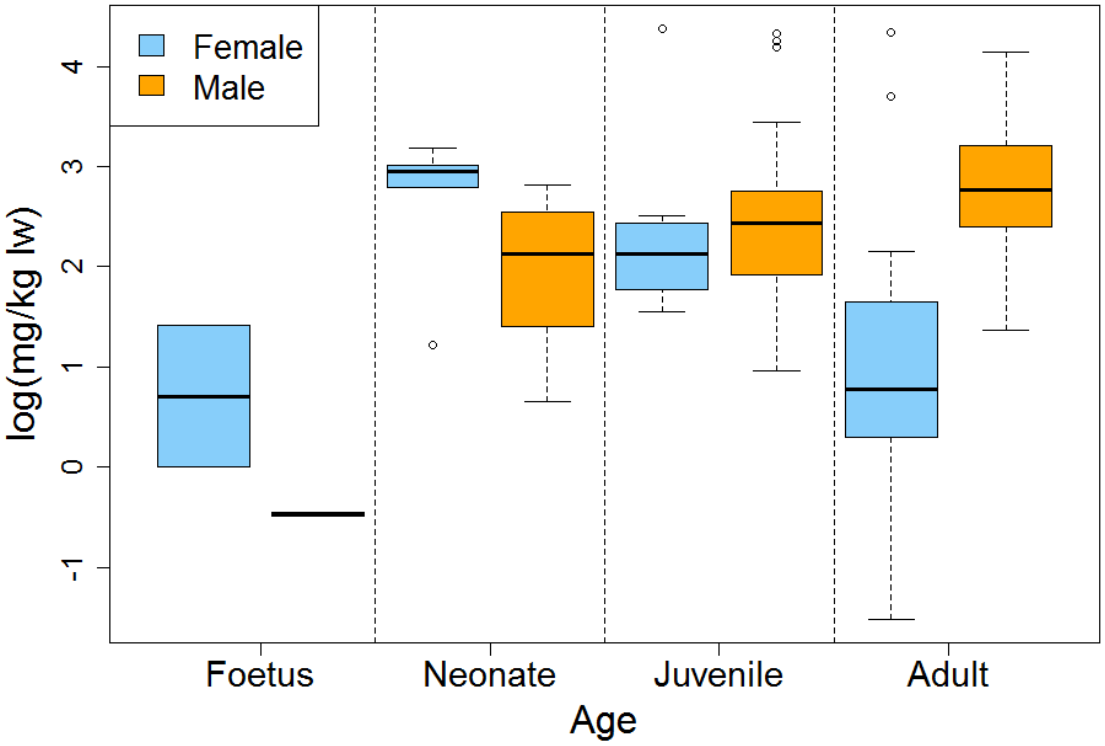

Figure G-1. *Tukey HSD post-hoc comparison for all PCB data in harbour porpoises (2006-2016).*

PCBs – selection NCC<4

Significant interaction term between age and sex ($p<0.05$).

Significant differences (Tukey HSD post-hoc comparison):

- M:A-F:A 0.0000049
- F:J-F:A 0.0013987
- M:J-F:A 0.0000000
- F:N-F:A 0.0000264
- M:N-F:A 0.0128129
- F:F-M:A 0.0243570
- M:F-M:A 0.0000418
- F:N-F:F 0.0499609

- F:J-M:F 0.0018344
- M:J-M:F 0.0001083
- F:N-M:F 0.0001111
- M:N-M:F 0.0037508

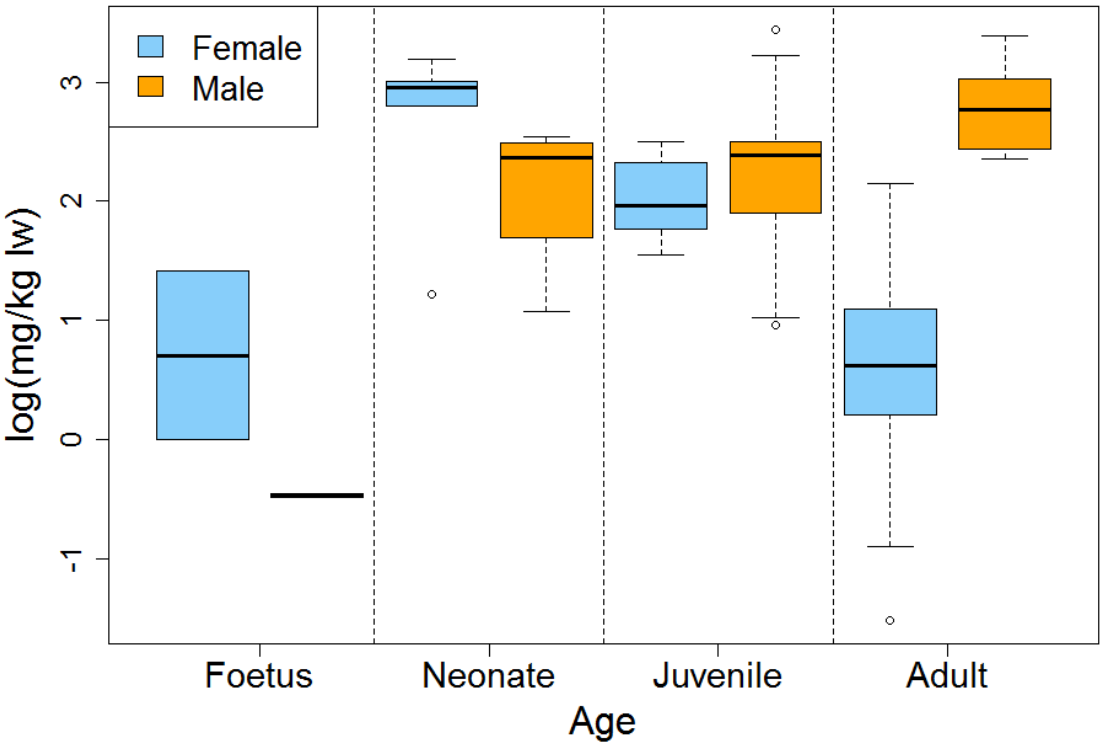

Figure G-2. *Tukey HSD post-hoc comparison for all PCB data (NCC<4) in harbour porpoises (2006-2016).*

PBDEs – all samples

No significant interaction term between age and sex (p=0.14).

Both Sex (p = 0.026) and Age (p<0.01) are significant

Sex

M-F 1. 1.42e-05

Age

F-A 0.0000010
J-A 0.0190687
N-A 0.4631152 (Not significant)
J-F 0.0000000
N-F 0.0040325
N-J 0.0195839

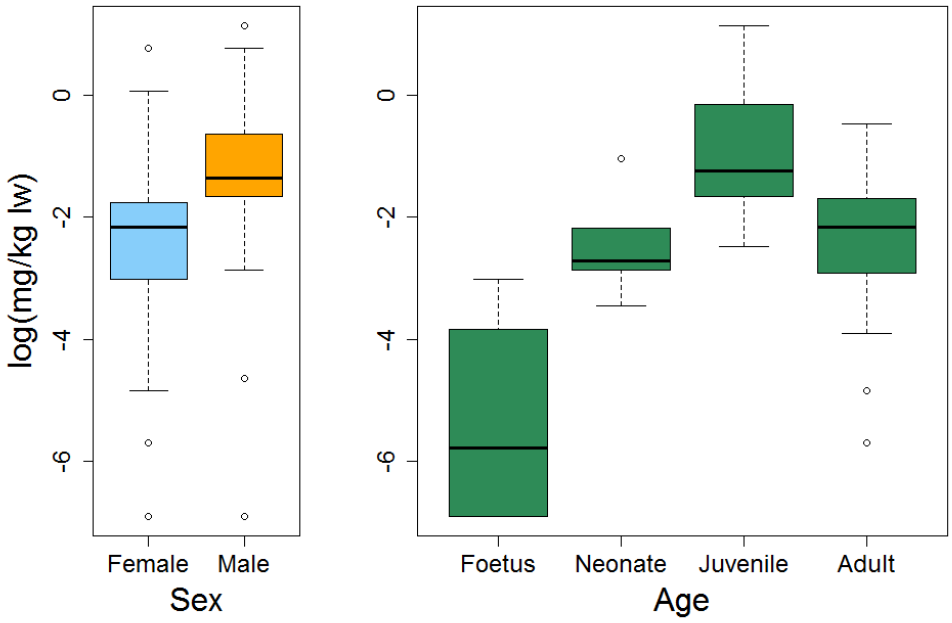

Figure G-3. *Comparison for all PBDE data in harbour porpoises (2006-2016) per sex and per age class.*

PBDEs – selection NCC<4

No significant interaction term between age and sex (p=0.053)

Both Sex (p = 0.01) and Age (p<0.01) are significant

- F-A 0.0000046
- J-A 0.0977518 not significant
- N-A 0.9993396 not significant
- J-F 0.0000000
- N-F 0.0077383
- N-J 0.7552182 not significant

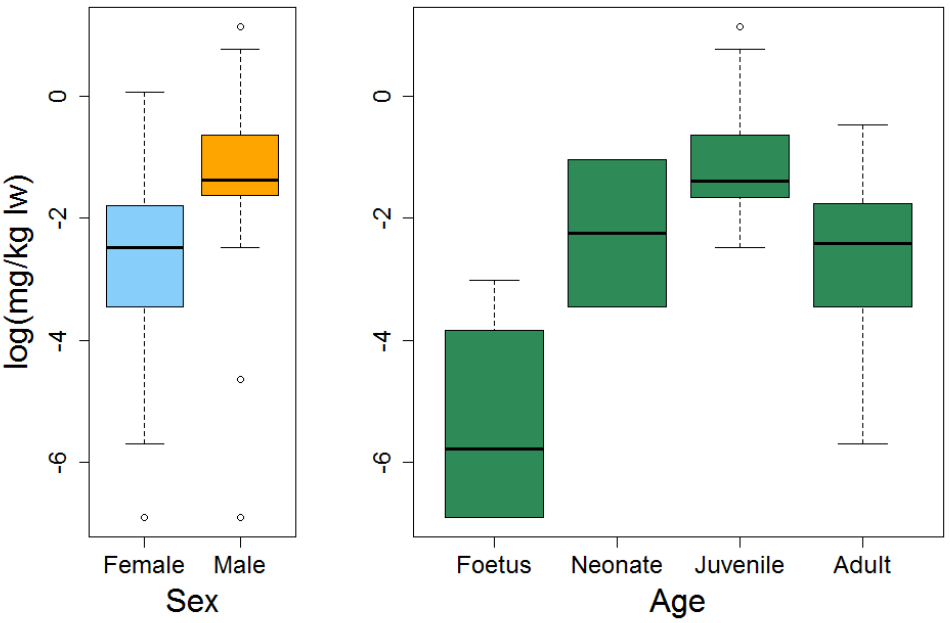

Figure G-4. *Comparison for PBDE data (NCC<4) in harbour porpoises (2006-2016) per sex and per age class.*

Annex H Number of samples per analysis

Number of samples per analysis – all samples

	PCBs	DI-PCBs	PBDEs	HCB	PFCs
Foetus – female	2	-	2	-	-
Foetus – male	2	-	2	-	-
Neonate – female	5	-	1	-	4
Neonate – male	10	-	4	-	4
Juvenile – female	8	-	8	-	8
Juvenile – male	31	-	28	-	26
Adult – female	26	14	26	10	10
Adult – male	7	7	7	7	7
TOTAL	**91**	**21**	**78**	**17**	**59**

Number of samples per analysis – samples with NCC>4

	PCBs	DI-PCBs	PBDEs	HCB	PFCs
Foetus – female	2	-	2	-	-
Foetus – male	2	-	2	-	-
Neonate – female	5	-	1	-	4
Neonate – male	4	-	1	-	1
Juvenile – female	7	-	7	-	7
Juvenile – male	25	-	24	-	22
Adult – female	19	5	20	4	4
Adult – male	5	5	5	5	5
TOTAL	**69**	**10**	**62**	**9**	**43**

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'To explore the potential of marine nature to improve the quality of life'

The Wageningen Marine Research mission
- To conduct research with the aim of acquiring knowledge and offering advice on the sustainable management and use of marine and coastal areas.
- Wageningen Marine Research is an independent, leading scientific research institute

Wageningen Marine Research is part of the international knowledge organisation Wageningen UR (University & Research centre). Within Wageningen UR, nine specialised research institutes of the Stichting Wageningen Research Foundation have joined forces with Wageningen University to help answer the most important questions in the domain of healthy food and living environment.