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Polluted porpoises: Generational transfer of organic contaminants in harbour porpoises from the southern North Sea

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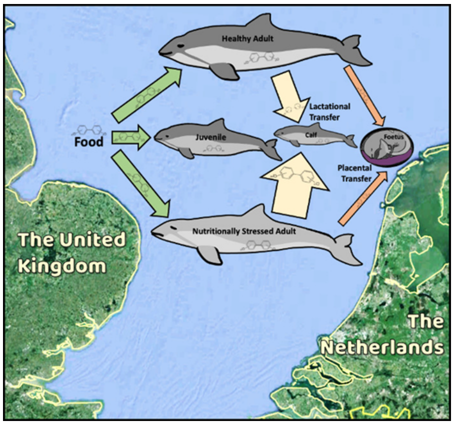
HIGHLIGHTS

- PCBs, PBDEs and HCB are transferred to next generations through placenta and milk.
- Placenta contained similar PCB and PBDE concentrations as foetus (blubber).
- Levels of PCBs and PBDEs increased after birth as a result of milk intake.
- Nutritional stress in adult females led to higher offloading in the milk.
- 92% of adult male porpoises had PCB levels exceeding a threshold for health effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs) and hexachlorobenzene (HCB), bioaccumulate in marine ecosystems. Top predators contain high levels of POPs in their lipid-rich tissues, which may result in adverse effects on their reproductive, immune and endocrine functions. Harbour porpoises (*Phocoena phocoena*) are among the smallest of cetaceans and live under high metabolic demand, making them particularly vulnerable to environmental pressures. Using samples from individuals of all maturity classes and sexes stranded along the southern North Sea ($n = 121$), we show the generational transfer of PCBs, PBDEs and HCB from adults to foetuses. Porpoise placentas contained 1.3–8.2 mg/kg lipid weight (lw) Sum-17PCB, <dl-0.08 mg/kg lw Sum-17PBDE and 0.14–0.16 mg/kg lw HCB, which were similar to concentrations in foetus blubber. Contaminant levels increased significantly after birth through suckling. Milk samples contained 0.20–33.8 mg/kg lw Sum-17PCB, 0.002–0.51 mg/kg lw Sum-17PBDE and 0.03–0.21 mg/kg lw HCB. Especially lower halogenated and more toxic contaminants were transferred to calves, exposing them to high levels of contaminants early in life. Of all animals included in this study, 38.5% had PCB concentrations exceeding a threshold level for negative health effects (>9 mg/kg lw). This was particularly true for adult males (92.3% >9 mg/kg lw), while adult females had relatively low PCB levels (10.5% >9

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mg/kg lw) due to offloading. Nutritional stress led to higher offloading in the milk, causing a greater potential for toxicity in calves of nutritionally stressed females. No correlation between PCB concentration and parasite infestation was detected, although the probability of a porpoise dying due to infectious disease or debilitation increased with increasing PCB concentrations. Despite current regulations to reduce pollution, these results provide further evidence of potential health effects of POPs on harbour porpoises of the southern North Sea, which may consequently increase their susceptibility to other pressures.

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1. Introduction

Exposure to chemical contaminants is one of the many stressors that may result in adverse effects on the health status of individual animals (Harrison et al., 1997; Aguirre and Tabor, 2008; McHuron et al., 2018). Known contaminants of concern to marine wildlife are the lipophilic persistent organic pollutants (POPs), which include polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD). These POPs are resistant to biological and physical degradation (McHuron et al., 2018), some with half-lives of up to a hundred years, and they bioaccumulate and biomagnify in marine food webs (Norstrom, 2002; Jepson et al., 2016; Murphy et al., 2018).

As relatively long-living top predators, marine mammals can accumulate high levels of POPs in their lipid tissue (Weijs et al., 2009; Desforges et al., 2018). As such, they have been the focus of many studies showing associations between POPs and impairment of reproductive, immune and endocrine functions (Reijnders, 1986; Murphy et al., 2015; Jepson et al., 2016; Desforges et al., 2016; Desforges et al., 2018; Williams et al., 2020a, 2020b; Williams et al., 2021). Exposure of mammalian species to contaminants occurs mainly through dietary intake (McHuron et al., 2018).

The effects of contaminants on the health status of harbour porpoises (*Phocoena phocoena*) in the North Sea have been a particular concern (Jepson et al., 2005; Hall et al., 2006a; Weijs et al., 2009; Murphy et al., 2015; IJsseldijk et al., 2018). Harbour porpoises are among the smallest of cetacean species with a relatively thick blubber layer compared to other small cetaceans (Worthy and Edwards, 1990). They live on an energetic knife-edge because they have a relatively large body surface to body volume ratio, a high metabolic demand and live in cold water environments (Murphy et al., 2015; Wisniewska et al., 2016). This makes them vulnerable to environmental pressures impacting their health and reproduction. The Northeast Atlantic houses a continuous population of harbour porpoises ranging from France to northern Norway (Fontaine et al., 2007). At the same time it is a hotspot of anthropogenic activities, with many coinciding and cumulative stressors (Halpern et al., 2008, 2015), resulting in high disturbance rates for the species living within these waters. High contaminant exposure in harbour porpoises from the North Sea area has previously been associated with high parasitic exposure (Bull et al., 2006; Pierce et al., 2008), increased prevalence of infectious diseases (Jepson et al., 2005; Hall et al., 2006a; Pierce et al., 2008; Mahfouz et al., 2014; Jepson et al., 2016) and reproductive failure (Farré et al., 2010; Murphy et al., 2015; Jepson et al., 2016). More recent studies suggest differences in chemical exposure during the life stages, which potentially negatively affect development and reproduction. Immature harbour porpoises were exposed to more neurotoxic mixtures of PCB congeners than adults (Williams et al., 2020a) and testes weights were negatively associated with PCB concentrations and nutritional stress (Williams et al., 2021).

In the southern North Sea, harbour porpoise stranding rates have significantly increased since 2005. This increase was most prominent along the coastline of The Netherlands (IJsseldijk et al., 2020). A shift in distribution of harbour porpoises from the northern to the southern North Sea was reported based on two large-scale international abundance surveys in 1994 and 2005 (Hammond et al., 2002, 2013). Although a range shift likely explains the majority of the increase in stranding numbers in the southern North Sea since the 1990s, stranding numbers have been excessively high since 2005, with several periods of temporal elevated mortality levels thereafter (IJsseldijk et al., 2020; IJsseldijk et al., 2021b). As the cause(s) of the increase in strandings and mortalities are yet to be determined, there is a need to assess the overall health status of harbour porpoises and their threats in this region.

The aims of this study were threefold: 1) to assess levels of exposure of PCBs, PBDEs, HCB and HCBD in stranded harbour porpoises from the southern North Sea across the different maturity classes and sexes, 2) to assess the importance of placental and lactational transfer of these compounds through the analyses of umbilical cord, placenta, milk and foetus samples and the influence of nutritional status on offloading via lactation, and 3) to investigate the relationship between exposure levels and health status using data on nutritional condition, health status, causes of death and parasite infestation gained from necropsies. This study represents the most recent and comprehensive investigation into generational cycling of persistent organic pollutants in an abundantly stranded small cetacean of the southern North Sea. We provide novel insights into the understanding of generational transfer of contaminants in cetaceans, the health status of harbour porpoises in the southern North Sea, and the threats that they face.

2. Material & methods

2.1. Collection

2.1.1. Post-mortem investigations

Between January 2006 and September 2019, more than 1800 stranded or bycaught harbour porpoises were collected in The Netherlands for post-mortem investigations, that took place at the Dutch Royal Institute of Sea Research on Texel in 2006 and 2007, and at the Faculty of Veterinary Medicine, Utrecht University, since 2008. Necropsies and tissue sampling were conducted following internationally standardised guidelines (IJsseldijk et al., 2019). For each case, stranding date, location, sex, total length (cm), weight (kg) and blubber thickness (mm) were recorded. Blubber thickness was measured immediately anterior to the dorsal fin at three locations (dorsal, lateral and ventral, in mm). Each case was assigned a nutritional condition code (NCC) which was visually assessed based on the dorsal musculature, presence/absence of visceral fat and through quantitative assessment of blubber thickness. NCC was appointed on a six-point scale with NCC1 representing very fat and muscular animals and NCC6 emaciated animals. These scores were later grouped with NCC1-2 = good condition, NCC3-4 = moderate condition and NCC5-6 = poor condition.

2.1.2. Sample selection

A total of 112 harbour porpoises that were stranded along the Dutch coast and 9 foetuses were analysed for contaminants, with the samples comprising both sexes and all maturity classes (Supplementary Table (STab) 1 and Supplementary Figure (SFig) 1). These cases were selected based on carcass freshness and, subsequently, the availability of samples for toxicological analyses. During necropsies, blubber samples were collected and stored in aluminium foil at −20 °C. Additionally, milk from 14 adult females was collected and stored in glass containers at −20 °C., and placenta (n = 3) and umbilical cord tissues (n = 2) of mother-foetus pairs were collected and stored at −20 °C.

2.1.3. Assessment of reproductive status

Following Murphy et al. (2010, 2015, 2018), ovarian samples of adult females in the study were assessed for the presence of ovarian corpora scars (corpus albicans or corpus luteum, CA and CL respectively). These samples were available for 20 of the 38 adult females. After macroscopic assessment, the ovaries were hand-sectioned into 0.5–2 mm slices and examined under a binocular microscope for the presence of additional ovarian corpora scars. Total numbers of ovarian corpora scars were counted, which is taken to represent the number of ovulations. Females were classified as sexually mature if they had one or more ovarian corpora scars. During the necropsies, pregnancy was established by the presence of a foetus. Lactation was assessed via gross examination of the mammary glands. Females were classified into six reproductive states: (1) sexually immature, (2) pregnant (foetus present), (3) pregnant and lactating, (4) sexually mature and lactating, (5) resting mature (not pregnant or lactating), and (6) suspected recently aborted (dilation of uterus outside the calving season).

2.2. Preparation

2.2.1. Health status categorisation

For harbour porpoises in a very fresh to moderately putrefied condition, the most likely cause of death was determined based on macroscopic and microscopic examination. Additionally, for all cases the parasite presence and severity of parasite infestation were scored for four major organs (lung, liver, stomach and middle ear/sinuses): 0 = none, 1 = mild, 2 = moderate and 3 = severe (Ten Doeschate et al., 2017). The porpoises included in this study were subdivided into two categories based on the most significant necropsy findings. The first category includes all individuals that most likely died as a result of bycatch (diagnosed following IJsseldijk et al., 2021a), due to a predatory attack (diagnosed following Leopold et al., 2015), due to sharp or blunt-forced trauma, and due to dystocia (birth problems of full-term foetus) and intestinal volvulus where no signs of significant disease were detected to explain the dystocia or intestinal volvulus. The second category included all individuals that showed signs of significant infectious disease, high parasite loads and/or (very) poor nutritional conditions, and lacked signs of aforementioned 'acute' causes of death (STab 1). Foetuses and neonates were excluded from analyses where cause of death and parasitism were used as explanatory variables.

2.2.2. Maturity class determination

All cases were categorised into a maturity class based on their total length. Individuals <91 cm in length, and which stranded during the peak calving period for the species (May–August), were classified as neonates or immatures. Individuals with total lengths between 91 and 130 cm were considered juveniles or immatures, and individuals with total lengths of over 130 cm were classified as adults or matures. For those individuals at around 130 cm, visual inspection of the reproduction organs was conducted to properly subdivide them into juvenile or adult age classes (Lockyer, 2007; Murphy et al., 2020). Further subdivision of mature females into reproductive status categories was done as described under Section 2.1.3. The in-utero unborn calves are referred to as foetuses. The gestation period in the species is estimated to last around 10 to 11 months (Learmonth et al., 2014) and the lactation period may be up to 10 months (Lockyer, 2003).

2.3. Sample analyses

2.3.1. Sample homogenisation

The epidermis and 'air-exposed' parts of the blubber samples were removed, and the remaining blubber sample was dissected into small pieces. The placenta and umbilical cord samples were homogenised using a blender prior to analysis.

2.3.2. Lipid content

Total extractable lipid (triglycerides and phospholipids) levels were determined in the blubber samples following the Bligh and Dyer (B&D) method, modified by De Boer (1988). In short, 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.

2.3.3. Chemical analyses

Samples were analysed using accelerated solvent extraction (ASE) and gas chromatography coupled to a mass spectrometry (GC–MS) method to quantify PCBs, PBDEs, HCB and HCBD. This method (SOP 2.10.3.050 Biota and environmental matrices: Determination of micro pollutants after ASE extraction and GC–MS detection) was validated for biota according to ISO 17025. Solvents and Florisil were obtained from LGC Standards Promochem. The samples were mixed with sodium sulphate and transferred to an ASE cell containing 25 g Florisil. The ASE cell was extracted three times using pentane/dichloromethane (85/15). After addition of 1 ml of isooctane as a keeper, the extract was concentrated to 1 ml in a rotary evaporator.

PCBs, PBDEs, HCB and HCBD were then determined by a GC–MS detector. PCBs, HCB and HCBD were measured using a Shimadzu 2010GC coupled with a Shimadzu QP2010 Ultra MS using an Electron Impact (EI) source. Separation was performed over 60 m (0.25 × 0.25) J&W HT-8 column (DaVinci Europe, The Netherlands, manufactured by SGE Analytical Science). Source and transfer line temperature were set to 300 °C. 5 μl sample was injected by Large Volume Injection (LVI). The oven program was as follows: start at 95 °C, hold for 3 min, 25 °C/min to 170 °C, 2.5 °C/min to 255 °C, hold for 10 min, 45 °C/min to 325 °C. *m/z* 256/258, 290/292, 326/324, 360/362, 394/396 and 428/430 were used as quantifier and qualifier ions for PCBs while 227/225 was used for HCBD and 286/284 were used for HCB.

PBDEs were measured using an Agilent 6890GC coupled with a 5973MS with NCI source and a 50 m (0.25 × 0.25) J&W CPsil8 column (DaVinci Europe, The Netherlands) for separation. Source and transfer line temperature were set to 200 and 290 °C respectively. 1 μl sample was injected by split/splitless injection at 275 °C. The oven program was: start at 90 °C, hold for 3 min, 30 °C/min to 210 °C, hold for 20 min, 5 °C/min to 290 °C, hold for 13 min, 30 °C/min to 325 °C and hold for 15 min. *m/z* 81 and 79 were used as quantifier and qualifier ions.

Chemical analysis was carried out according to ISO17025 accredited methods with full quality control procedures such as a blank and reference sample (eel filet). For compounds that were detected in a blank sample the limit of quantification was set to 5× blank value. Compounds in the control sample were plotted in Sheward quality charts and results were required to be within the 2 s. Also the lab participates yearly in proficiency testing with satisfactory scores in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) proficiency test scheme. MDL and precision for each compound is available in the supplementary information (STab2). Calibration was performed using certified standards (AccuStandard, The Netherlands) and at least a six-point calibration curve with points between 0.5–1000 ng/ml and $r^2 > 0.99$. Concentrations below the detection limit were reported as <dl ≤ detection limit.

A maximum of 28 PCB congeners could be quantified based on the calibration standards, whereas 20–24 PCB congeners were actually detected in the samples. For PBDEs 17 congeners could be quantified based on the calibration standards, and up to 12 congeners were detected in the samples. For the comparison of PCB, PBDE, HCBD and HCB concentrations between harbour porpoises and between different sample types, concentrations were calculated to 100% lipid per sample (mg/kg lipid weight (lw)) using the lipid content of each sample.

2.3.4. Analyses with respect to threshold levels

A Sum-17PCB, based on the most relevant PCB congeners, consisted of congener # 47, 49, 52, 101, 105, 118, 128, 138, 149, 151, 153, 156, 170, 180, 187, 194, and 202. To enable a comparison with the International Council for the Exploration of the Sea (ICES) Sum-PCB, a Sum-7PCB was calculated using congener # 28, 52, 101, 118, 138, 153, and 180.

A Sum-17PBDE, based on the predominant PBDE congeners in the samples, consisted of congener # 28, 47, 49, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154(+153), 183, 190, and 209. To allow comparison with the Environmental Quality Standards (EQS) within the Water Framework Directive (WFD; European Commission, 2013), an additional sum-parameter was selected, and sample concentrations were based on wet weight. This Sum-6PBDE consisted of congeners 28, 47, 99, 100, 153 and 154.

A first risk assessment for PCB exposure in cetaceans was carried out using earlier developed threshold levels for summed PCB concentrations (Kannan et al., 2000; Murphy et al., 2015; Jepson et al., 2016). These threshold levels were based on laboratory results and field studies of a variety of mammalians (for instance Helle et al., 1976; Murphy et al., 2015). The first one consisted of a threshold concentration of 9 mg/kg lw Sum-23PCB (congener # 95, 101, 110, 118, 128, 136, 138, 141, 144, 149, 151, 153, 170, 171, 174, 177, 180, 183, 187, 195, 201, 202, and 203) for the onset of physiological effects in marine mammals in general, based on studies assessing immunological and reproductive effects in seals, otters, and mink (Kannan et al., 2000; Jepson et al., 2016). A second threshold level consisted of a concentration of 11 mg/kg lw Sum-25PCB (congener # 18, 28, 31, 44, 47, 49, 52, 66, 101, 105, 110, 118, 128, 138, 141, 149, 151, 153, 156, 158, 170, 180, 183, 187, 194) for potential reproduction failure in resting adult female harbour porpoises (Murphy et al., 2015). Although female harbour porpoises with concentrations above this threshold may still become gravid, they probably did not offload their contaminant burdens due to either foetal or new-born mortality (Murphy et al., 2015). A third threshold of 41 mg/kg lw Sum-23PCB (congener # 95, 101, 110, 118, 128, 136, 138, 141, 144, 149, 151, 153, 170, 171, 174, 177, 180, 183, 187, 195, 201, 202, and 203) was used based on profound reproductive impairment in Baltic ringed seals (*Pusa hispida*) (Helle et al., 1976; Jepson et al., 2016).

Summed PCB concentrations, however, do not take into account individual toxicity of PCB congeners and chemical profiles may differ between e.g. age classes and sample types (Williams et al., 2020a). Therefore, the chemical profiles of the analysed congeners were also assessed from another perspective, grouping the PCB congeners in two ways. Grouping per degree of chlorination (tri-PCBs, tetra-PCBs, etcetera) provided information on the lipophilicity of a congener, which increases with a higher number of chlorine atoms attached (Safe and Hutzinger, 1984). As metabolism is structure dependant, PCBs were also grouped into six Structure Activity Groups (SAGs) based on their capacity to be biotransformed (Boon et al., 1994; Boon et al., 1998; Cullon et al., 2012). SAGs I-II-V are considered highly persistent in cetaceans, whereas SAGs III-IV-VI may be metabolised to a certain extent dependant on the species (Boon et al., 1994; Cullon et al., 2012) (STab 3).

For PBDEs, HCB and HCBD no thresholds for effects on marine mammals have been established. The WFD uses the following EQS for these compounds in biota: 0.0085 μg/kg wet weight (ww) Sum-6PBDE (see above), 10 μg/kg ww HCB, and 55 μg/kg ww HCBD (European Commission, 2013). However, the EQS for these contaminants were defined for fish and the relevance of the EQS for higher trophic levels, such as harbour porpoises remains unexplored.

2.4. Data analyses

Data exploration was applied for PCB and PBDE data following the method described by Zuur et al. (2009) prior to analysis. As for HCBs only 47 individuals (6 foetuses, 6 neonates, no juveniles, 22 adult females, 13 adult males) were analysed, this sample size was considered too small for an extensive data analysis. Data exploration, visualisation and analyses were performed using R version 3.6.3 (R Core Team, 2017), with package ggplot2 (version 3.3.2), grid (version 3.6.3) and gridExtra (version 2.3). An overview of statistical models is given in STab 4, with descriptions in the following subsections.

2.4.1. Generational transfer of PCB and PBDE

A Generalized Linear Model (GLM) was used to assess the log-Sum17PCB exposure in male and female harbour porpoises of different maturity classes (Model 1). The log-Sum17PCB was analysed in relation to the independent indicator variables: sex, maturity class, the interaction between sex and maturity class, and the log-Sum17PBDE. Seven individuals had missing data and were excluded from this analysis. Model selection was undertaken by a stepwise backward selection using Akaike Information Criterion (AIC), where a value difference of >2 was considered an improved model. Residuals were checked for normality by normal probability (QQ) plots. For the variables in the final model, 95% (log-)likelihood profile confidence intervals were calculated.

The average proportion of highly persistent (SAGs I-II-V; STab 3) and less persistent (SAGs III-IV-VI; STab 3) PCBs in blubber was assessed over total length of the harbour porpoises using a linear model (Model 2) and 95% confidence intervals of the model as well as the correlation coefficient (R-squared) were calculated. Additionally, the mean proportion per maturity class was calculated to visualize the PCB and PBDE profiles.

Partial correlation analysis, corrected for maturity class, sex and the interaction between maturity class and sex, was performed for log-Sum-17PCB and log-Sum-17PBDE (Model 3).

2.4.2. Contaminant concentration in adult females

To assess the relation between ovarian corpora scars, porpoise total length, log-Sum17PCB and log-Sum17PBDE concentrations in adult females, a GLM with a Poisson distribution was used with the number of ovarian scars as the dependent variable, and the reproductive status of the female as independent indicator variable (five categories: lactating, pregnant, pregnant and lactating, resting and suspect aborted), total length, log-Sum17PCB in blubber, log-Sum17PBDE in blubber, and an interaction between the reproductive status and total length (Model 4). Lastly, two linear models were used to assess the log-Sum17PCB and log-Sum17PBDE concentrations in milk in relation to total length (Model 5 and 6, respectively).

2.4.3. PCB and PBDE exposure in relation to health parameters

To assess relationships between log-Sum17PCB and necropsy findings as proxies of the health status of the harbour porpoises, a GLM was used (Model 7). The log-Sum17PCB was analysed in relation to the independent indicator variables: maturity class, sex, log-Sum17PBDE, cause of death (two categories: physical trauma and other acute causes versus infectious disease and/or debilitated animals), nutritional condition (NCC, three categories: good, moderate or poor), the four organs scored for parasite infestation (lung, liver, stomach, ear), and an interaction between maturity class and sex, and an interaction between cause of death categories and NCC. Model selection was done by a stepwise backward selection using AIC, where a value difference of >2 was considered an improved model. Residuals were checked for normality by normal probability (QQ) plots. For the variables in the final model, 95% (log-)likelihood profile confidence intervals were calculated.

A binomial logistic regression (Model 8) was conducted to assess the relationship between the Sum17PCB and the cause of death categories of the harbour porpoises. All animals that mostly likely died as a result of infectious disease and/or debilitation were given a score 1 and all animals that most likely died due to physical trauma or other acute causes were given a score 0. Log odds ratios given, and 95% (log-)likelihood profile confidence intervals were calculated.

3. Results

3.1. Contaminant concentrations and profiles in maturity classes

3.1.1. Generational transfer of contaminants

Contaminant concentrations in blubber samples of harbour porpoises ranged from 0.21–90.15 mg/kg lw for Sum-17PCB, <dl-3.24 mg/kg lw for Sum-17PBDE and < dl-0.42 mg/kg lw for HCB (STab 1, STab 5). Concentrations of HCBD were below the detection limit (<0.1 μg/kg) in all analysed samples, except when specifically mentioned. Lowest mean contaminant concentrations were detected in blubber samples of foetuses and adult females (Fig. 1). No clear differences in mean concentrations of PCBs and PBDEs were observed between neonates and juveniles. No comparison could be made for HCB as no chemical analysis was performed on samples of juvenile harbour porpoises in this study.

Sum-17PCB concentrations were significantly higher in adult males compared with adult females. All independent indicator variables remained in the final model; maturity class, sex, an interaction between maturity class and sex, and the log-Sum17PBDE (Model 1, AIC = 253.6). 95% (log-) likelihood profile confidence intervals for Sum-17PBDE were 0.60;0.88, for neonates were 0.21;1.61 and for adult males were 0.32;1.70: indicating strong, positive relationships. All 95% (log-) likelihood profile confidence intervals can be found in STab 6.

3.1.2. PCB and PBDE profiles

The dominant PCB congener groups in the blubber samples of harbour porpoises were the hexa PCBs followed by the penta and/or hepta PCBs (Fig. 2, SFig 2). Foetuses, neonates and juveniles contained a higher proportion of lower chlorinated PCBs (tri, tetra and penta) than adult porpoises. Adult males had the highest proportion of hexa and hepta PCBs compared to the other maturity classes, whereas adult females had the highest proportion of octa PCBs compared to the other maturity classes.

Based on Structure Activity Groups (SAGs), the highly persistent PCBs (SAGs I-II-V) showed a significant increase in proportion per

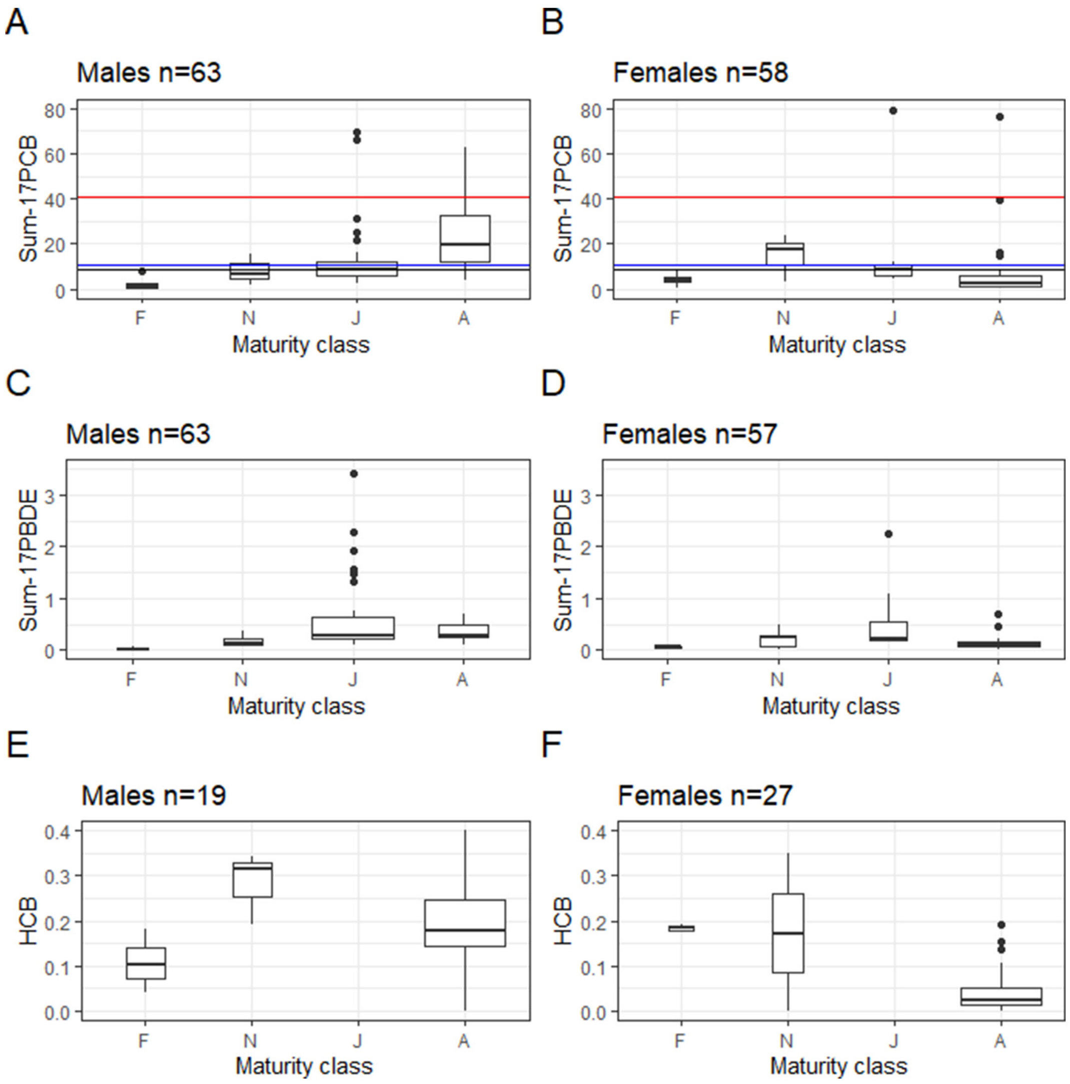

Fig. 1. Average contaminant concentrations (mg/kg lw) in blubber samples of harbour porpoises stranded along the Dutch coast in 2006–2019 per maturity class. In the left panels the males and in the right panels the females. F = foetus, N = neonate, J = juvenile and A = adult. Upper panels (A and B): Sum-17PCB, including threshold levels of 9 mg/kg lw (horizontal black line), 11 mg/kg lw (horizontal blue line) and 41 mg/kg lw (horizontal red line). Middle panels (C and D): Sum-17PBDE. Lower panels (E and F): HCB (no data available for juveniles). The black dots represent the outliers in the dataset. The width of the bars represents the sample size, the horizontal lines the medians, the boxes the first to third quartile, and the tails the minimum and maximum. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

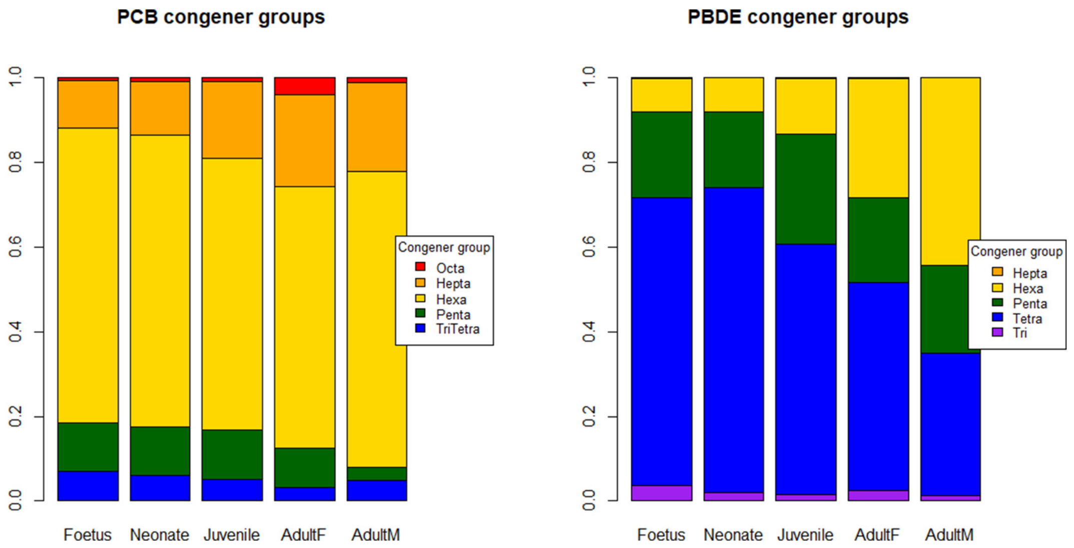

Fig. 2. Average proportion of PCB (left panel) and PBDE (right panel) concentrations per congener group in blubber of all maturity classes of harbour porpoises stranded along the Dutch coast in 2006–2019 (n = 120). AdultF = adult females, AdultM = adult males. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

total length (95%CI 113.33;277.35), whereas for the less persistent PCBs (SAGs III-IV-VI) a significant decrease in proportion was observed with length (95% CI −277.35;−113.33) (Fig. 3). The increase of highly persistent PCBs was mainly based on a build-up of SAG I PCBs in the adult porpoises, together with a decrease of the less persistent PCBs in the adult porpoises (SFig 3).

The most dominant PCB congeners in both adult females and foetuses were the hexa PCBs (predominantly CB-138, CB-149 and CB-153) and hepta PCBs (CB-187). The relative PCB congener profiles of the foetus differed from its mother, as a higher proportion of lower chlorinated PCBs was observed in the foetus compared to its mother (SFig 4). Hexa PCB levels in the foetus and its mother were similar (relative value of 1). Tri PCBs were often below detection limit and therefore excluded from this comparison.

Dominant PBDE congener groups were tetra PBDEs, followed by penta and/or hexa PBDEs (Fig. 2, SFig 6). Foetuses and neonates had the highest proportion of tetra PBDEs, whereas the proportion of tetra PBDEs decreased in juveniles and adults which in turn showed a higher proportion of hexa PBDEs (Fig. 2). Adult males had the highest proportion of hexa PBDEs compared to the other maturity classes, whereas the proportion of penta PBDEs was relatively similar in all classes.

PBDE patterns in all maturity classes showed a large variation (SFig 6). The relative proportion, however, showed a similar relation for mother and foetus as for PCBs, with a decreasing transfer of PBDEs to

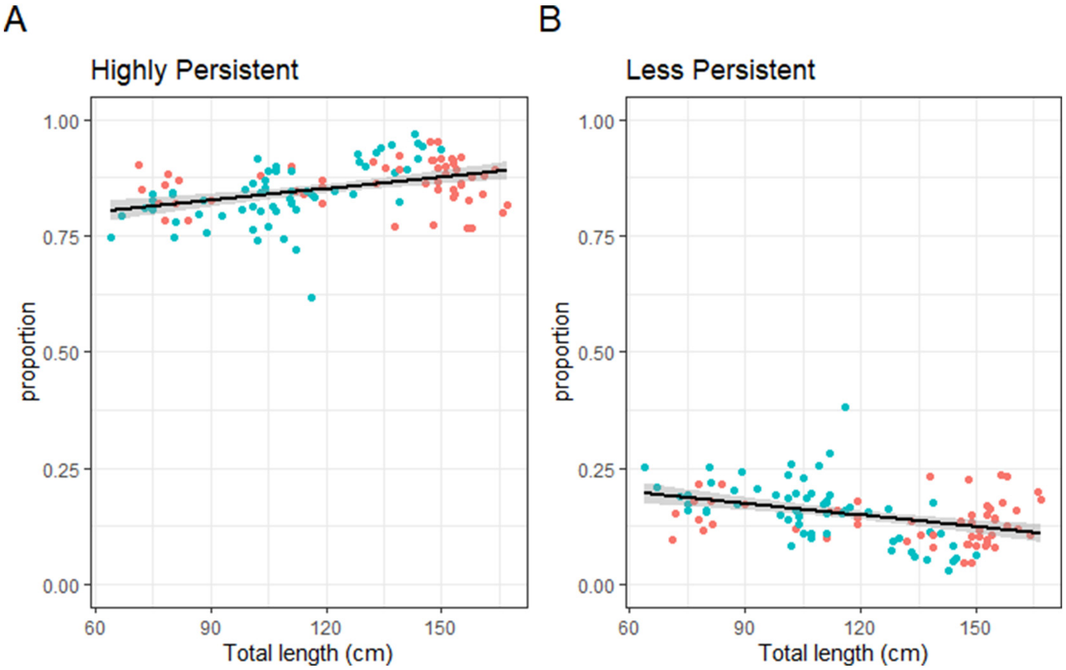

Fig. 3. Average proportion of highly persistent (SAG groups I-II-V; left panel) and less persistent (SAG groups III-IV-VI; right panel) PCBs in blubber of all maturity classes (here expressed as total length) of harbour porpoises stranded along the Dutch coast in 2006–2019 (n = 120) (Multiple R-squared: 0.1646). Females plotted in pink, males in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the foetus with increasing bromination (SFig 7). Foetuses contained a higher proportion of tetra PBDEs, whereas hexa PBDEs were more retained in the mother (SFig 7).

3.1.3. Partial correlation analysis Sum17PCB and Sum17PBDE

Sum-17PCB and Sum-17PBDE concentrations in stranded harbour porpoises were strongly correlated (Model 3, correlation coefficient (R) = 0.701) (n = 114) (Fig. 4).

3.2. Contaminant concentrations in milk, placenta and umbilical cord

Concentrations of Sum-17PCB in milk samples ranged between 0.20 and 33.8 mg/kg lw with a mean concentration of 8.6 mg/kg lw (n = 14) (Fig. 5A). No linear clear relation between PCB concentration in milk and total length was found, although smaller females tended to have higher PCB levels in their milk than larger females (Model 4, 95% profile likelihood interval slope of −0.142-0.046). The main PCB congener group in milk was formed by the hexa PCBs followed by the hepta PCBs (SFig 9). PCB profiles in milk resembled the PCB profiles in the blubber of the adult females (SFig 9). The proportion of Hepta and Octa PCBs in milk was slightly higher compared with the foetus, and the proportion of Tri/Tetra, Penta and Hexa PCBs in milk slightly lower (SFig 5).

Concentrations of Sum-17PBDE in milk samples varied between 0.002 and 0.51 mg/kg lw with an average concentration of 0.18 mg/kg lw (n = 14) (Fig. 5B). No relation between PBDE concentrations in milk and total length was found, although smaller-sized females also seemed to have higher PBDE concentrations (Model 5, 95% profile likelihood interval slope of −0.173-0.057). Main PBDEs in milk consisted of Tetra PBDEs followed by Penta and Hexa PBDEs (SFig 9). The proportion of hexa PBDEs in milk was slightly higher compared with the foetus, whereas the proportion of Tri/Tetra PBDEs was a little lower in milk (SFig 8).

For both Sum-17PCB and Sum-17PBDE a higher concentration in blubber was observed in combination with a higher concentration in milk (Fig. 5C and D).

HCB concentrations in milk samples ranged between 0.03 and 0.21 mg/kg lw. Where analysed, HCBD concentration in milk samples were below detection limit.

Concentrations in placenta (n = 3) were 1.3–8.2 mg/kg lw for Sum-17PCB, <dl-0.08 mg/kg lw for Sum-17PBDE, 0.14–0.16 mg/kg lw for HCB and < dl for HCBD (lipid % of 0.7–1.1). Concentrations in samples of the umbilical cord (n = 2) were 0.1–1.4 mg/kg lw for Sum-17PCB, 0.11–0.26 mg/kg lw for Sum-17PBDE, and < dl for both HCB and HCBD (lipid % of 0.5–0.7%). PCB, PBDE and HCB concentrations in the placenta samples were comparable to concentrations found in the blubber of the foetuses when based on lipid weight (although the lipid content of the placenta was low: 0.9% ± 0.2% lipid).

Individuals with a good nutritional condition (NCC 1–2) appeared to have higher milk lipid % (40.2–67.5) (n = 3) than individuals with a moderate and poor nutritional condition (NCC 3–6; milk lipid % of 7.6–50.6%; n = 11) (SFig 10A, STab 1 and STab 7). As the total number of milk samples was relatively low (n = 14), differences could not be tested on significance.

Sum-17PCB concentrations in the milk of adult females were lower in individuals with a good and moderate nutritional condition compared to that in individuals with a poor nutritional condition (SFig 10B). Adult females in good nutritional condition had on average 3.3 mg/kg lw Sum-17PCB in their milk (n = 3, range: 0.2–8.5 mg/kg lw), whereas individuals in moderate nutritional condition had an average concentration of 5.6 mg/kg 17PCB lw (n = 5, range: 1–17.6 mg/kg lw), and emaciated adult females had on average 13.7 mg/kg lw 17PCB in their milk (n = 6, range 4.7–33.7 mg/kg lw).

Sum-17PBDE concentrations in milk of adult females in good and moderate nutritional condition were lower than those in poor

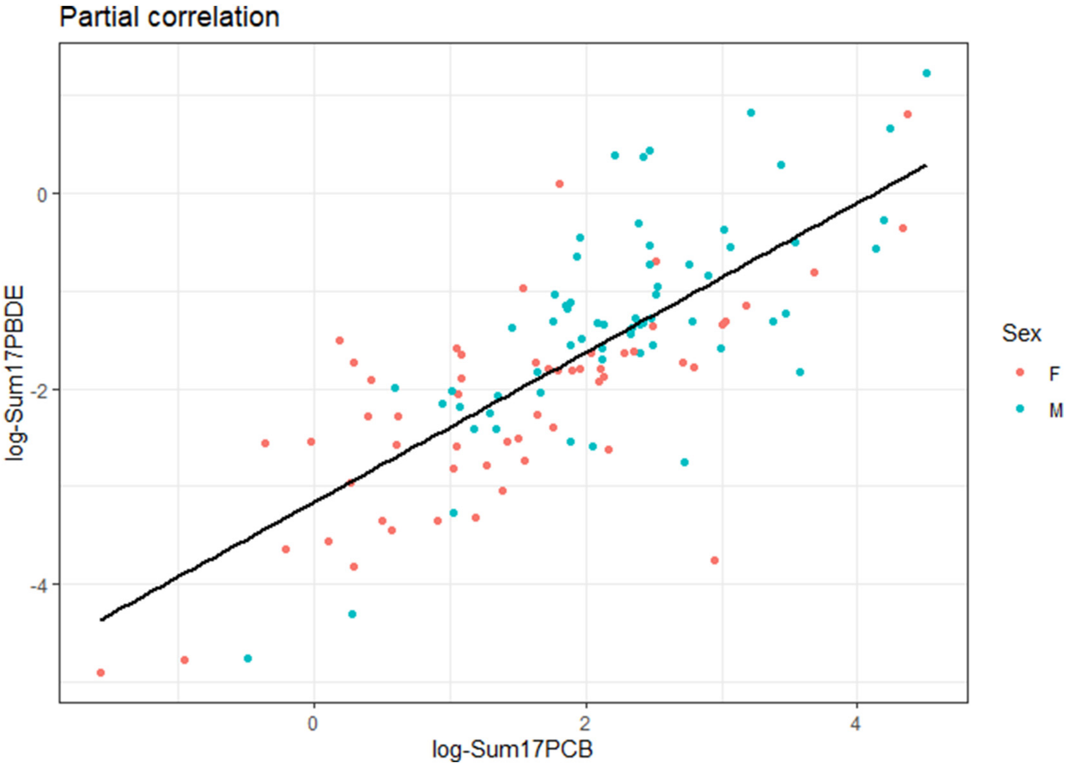

Fig. 4. Correlation between log-Sum17PCB and log-Sum17PBDE concentrations in blubber samples of harbour porpoises stranded along the Dutch coast in 2006–2019 (mg/kg lw, n = 114), with a correlation coefficient of 0.70. F = females (pink), M = males (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

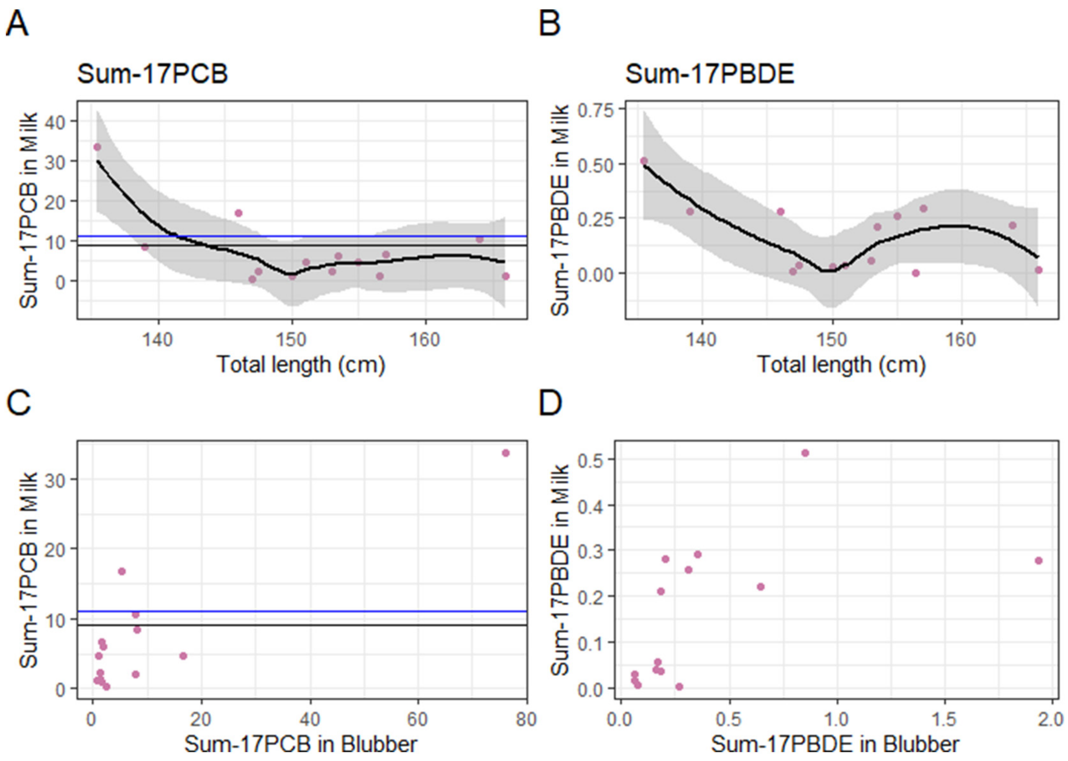

Fig. 5. A. Sum-17PCB concentrations in milk per total length (in cm) in adult females (mg/kg lw). B. Sum-17PBDE concentrations in milk per total length (in cm) in adult females. C. Sum-17PCB concentration in milk per Sum-17PCB in blubber of the same adult females. D. Sum-17PBDE concentration in milk per Sum-17PBDE concentration in blubber of the same adult females. Milk samples were derived from adult female harbour porpoises stranded along the Dutch coast in 2006–2019. For PCB graphs (A,C), threshold levels are indicated at 9 mg/kg lw (horizontal black line) and 11 mg/kg lw (horizontal blue line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

nutritional condition (SFig 10C). Sum-17PBDE concentrations in milk of adult female in good nutritional condition were on average 0.11 mg/kg lw ($n = 3$; range 0.006–0.28 mg/kg lw). Concentrations in animals in moderate condition were on average 0.08 mg/kg lw ($n = 5$, range 0.002–0.21 mg/kg lw). Sum-17PBDE concentrations in the milk of emaciated adult females contained an average concentration of 0.29 mg/kg lw ($n = 6$, range 0.18–0.51 mg/kg lw).

There was no clear relationship between early and late lactation and percentage of lipid of the milk samples when assessed over time; the percentage of lipid in milk was highly variable and ranged from 7.6–67.5% (SFig 11A). PCB and PBDE concentrations in milk varied throughout lactation, but on average were highest during early lactation (May–August) compared to late lactation (September–February) (SFig 11B–C). During early lactation, the median PCB concentration in milk was 9.89 mg/kg lw (range of 4.67–17.55 mg/kg lw), and the median PBDE concentration was 0.26 mg/kw lw (range of 0.13–0.29 mg/kw lw) ($n = 7$). During late lactation, the median PCB concentration in milk was 1.29 mg/kg lw (range of 0.20–33.76 mg/kg lw), and the median PBDE concentration 0.03 mg/kg lw (range of <0.01–0.51 mg/kg lw) ($n = 7$).

3.3. Potential effects of contaminants on health and reproduction

3.3.1. Threshold levels for negative effects

Of all harbour porpoises, 38.8% (47 out of 121) had PCB concentrations exceeding the 9.0 mg/kg lw Sum-PCB threshold for the onset of physiological endpoints in marine mammals (STab 8). While concentrations in foetuses were all below this threshold, 56.3% of the neonates and 48.9% of the juveniles had PCB concentrations exceeding this threshold. Whereas relatively few adult female harbour porpoises exceeded this threshold level (10.5%), almost all adult males did (92.3%).

Of all analysed porpoises, 33.1% had PCB concentrations above the threshold for potential reproduction failure in resting adult female harbour porpoises of 11 mg/kg lw (STab 8). As this threshold was mainly derived for resting adult females, a comparison is best made with this maturity class. In 10.5% of the adult females PCB concentrations were above the threshold level.

When applying the highest threshold level for profound reproductive impairment as derived from Baltic ringed seals (41 mg/kg lw Sum-PCB), 6.6% of the harbour porpoise samples exceeded this threshold level, which comprised mainly of adult males (15.4%), juveniles (both sexes), and, to a lesser extent, adult females (STab 8). Foetuses and neonates did not contain Sum-PCB concentrations above this threshold.

Most individuals had Sum-6PBDE concentrations well above the EQS for PBDEs of the WFD in all sample types, apart from a few where PBDE congener levels were all below the detection limit (STab 9).

3.3.2. Health parameters

We assessed whether the variables cause of death (two categories: physical trauma and other acute causes, and infectious disease and/or debilitated animals), NCC and parasite load in the four scored organs were associated with log-Sum17PCB. Cause of death and NCC were retained in the final model, but not parasite load in each of the four organs. The difference in the AIC of the model including or excluding the interaction between cause of death and NCC was <2 ($AIC = 198.84$ versus $AIC = 197.61$). The optimal model therefore included: sex, maturity class, log-SumPBDE, cause of death, NCC, the interaction between sex and maturity class, and the interaction between cause of death and NCC (for model selection, see STab 10).

The binomial logistic regression model (Model 7, Fig. 6) showed that the probability of dying from infectious disease and/or debilitation increased with higher PCB concentrations in the blubber. Log odds ratios

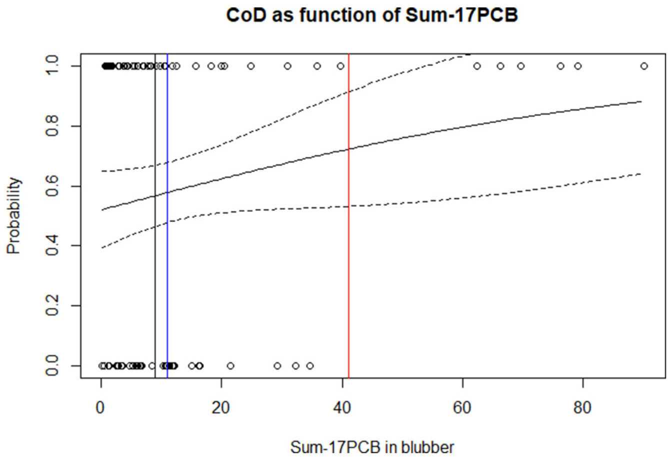

Fig. 6. Sum-17PCB concentrations in blubber as a function of 'cause of death', which is categorised as 'physical trauma and other acute causes' (plotted on the y-axis as 0) or 'infectious disease and/or debilitated' (plotted on the y-axis at 1). The probability of dying from physical trauma or other acute causes is the same as the probability of dying due to infectious disease and/or debilitation at low levels of Sum-17PCB (probability of 0.5). With increasing PCB, a positive correlation can be observed, meaning that the probability of dying due to infectious disease and/or debilitation is increasing. Note the wide 95% confidence interval range (dashed lines) as a result of small number of cases with Sum-17PCB concentrations >20. Threshold levels are indicated at 9 mg/kg lw (vertical black line), 11 mg/kg lw (vertical blue line) and 41 mg/kg lw (vertical red line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were −0.004;0.055, indicating that our data could not confirm a clear relationship. It is apparent that outliers in the dataset (those animals with Sum-17PCB in blubber of >40 mg/kg lw) strongly influenced these results (Fig. 6).

3.3.3. Reproductive organs and contaminant concentrations in adult females

The number of ovarian corpora scars in adult females increased with total length (Fig. 7). The smallest adult females (length between 130 and 140 cm) had 1–3 corpora scars (n = 3), while individuals with a total length of >140 cm had 4–17 corpora scars (n = 17). Pregnant females had 1–7 ovarian corpora scars (n = 6), (pregnant and) lactating females had 4–15 corpora scars (n = 5), females with suspected abortions had 9 and 12 corpora scars (n = 2), and resting females had more than 14 corpora scars (n = 4).

Pregnant females were in a better nutritional status than lactating females. Pregnant females had, on average, an NCC of 2.8 (n = 9), compared to 4.3 (n = 11, excluding pregnant females) in lactating females, and 5.7 (n = 3) in resting females.

The optimal model with the number of corpora scars as dependent variable, included the reproductive status, log-Sum17PCB in blubber, log-Sum17PBDE in blubber and total length, and not the interaction between reproductive status and total length (Model 8, AIC = 101.26). However, evaluation of the 95% CI of the most optimal model showed that only the reproductive status 'pregnant' was negatively correlated with increasing ovary scars (95% CI: −1.26- −0.08), while no significant relationship between the other parameters and number of corpora scars could be identified.

The number of ovarian corpora scars in adult females showed no correlation with either Sum-17PCB and Sum-17PBDE concentration in blubber samples (Fig. 8). There were two individuals, for which ovarian assessment was conducted, with a Sum-17PCB concentration above the thresholds of 9 mg/kg lw Sum-23PCB and 11 mg/kg lw Sum-25PCB (Fig. 8). The first individual was pregnant, had one ovary scar and a Sum-17PCB of 15 mg/kg lw in the blubber sample (see SText: case id UT1581). It had thus not yet been offloading. The second female was resting and had 15 ovary scars and a Sum-17PCB of 39.7 mg/kg lw in her blubber (see SText: case id UT1470). This strongly indicates that this female was unsuccessful in previously offloading, which based on Murphy et al. (2015) may be due to not successfully carrying a foetus to term, and/or the neonate dying soon after birth.

Recent abortion was suspected for two other adult females (Figs. 7–8). One had a severely dilated cervix, large asymmetry of the uterine horns, haemorrhage of the cervical wall and an endometritis. This individual stranded in April (2017) and did not show signs of lactation. The second female also had a severely dilated cervix, large asymmetry of the uterine horns and histologically oedema. This female live-stranded in May (2017), did not show signs of lactation, and no calf was observed at the time of stranding. Both animals had a CL on the left ovary and with a Sum-17PCB concentration < 9 mg/kg lw they likely previously offloaded successfully. The other sixteen adult females for which ovaria were assessed had a Sum-17PCB <9 mg/kw lw. No signs of reproduction failure were detected in these individuals, indicating successful offloading through pregnancy and (previous) lactation.

Among the adult females of which no ovarian assessment was conducted, another two individuals had PCB levels above the thresholds of 9 mg/kg lw Sum-23PCB and 11 mg/kg lw Sum-25PCB. One pregnant and lactating female had a Sum-17PCB concentrations of 16.4 mg/kg lw

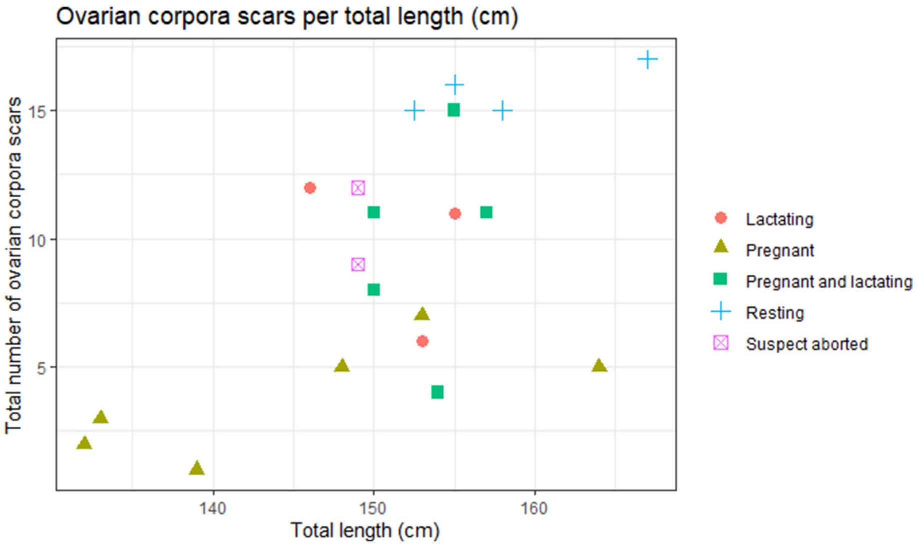

Fig. 7. The number of ovarian corpora scars (y-axis) per total length (x-axis) of adult female harbour porpoises that stranded along the Dutch coast in 2006–2019, grouped per reproductive class (n = 20). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

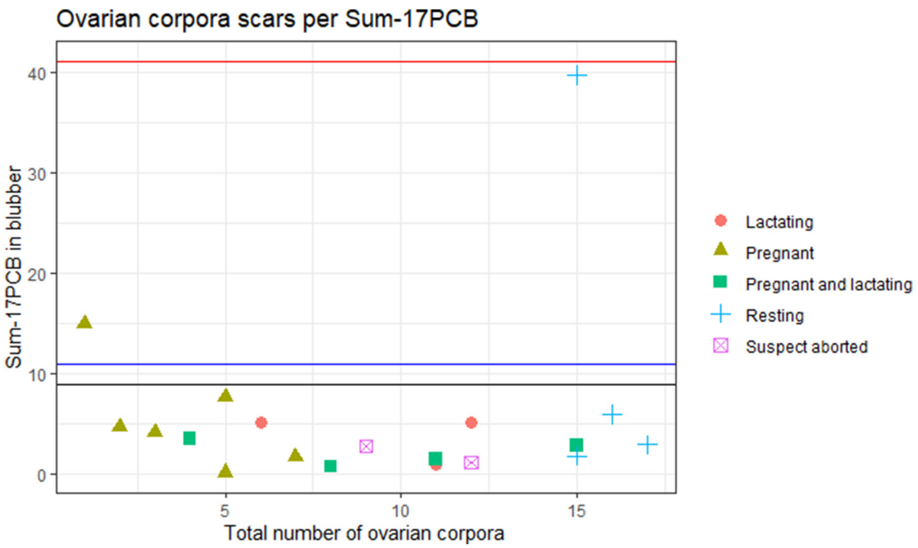

Fig. 8. Sum-17PCB per total number of ovarian corpora scars among the adult females that stranded along the Dutch coast in 2006–2019, grouped per reproductive class (n = 20; two green squares overlap at 11 ovarian corpora, cf. Fig. 7), including threshold levels of 9 mg/kg lw (horizontal black line), 11 mg/kg lw (horizontal blue line) and 41 mg/kg lw (horizontal red line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in her blubber and 17.6 mg/kg lw in her milk. The individual died due to dystocia (problems during birth) and was actively lactating for the impending birth. PCB mobilisation in the milk was apparent, but offloading had not yet occurred (see SText: case id UT1756). The other individual, an emaciated but lactating female, had 76.2 mg/kg lw in her blubber and 33.8 mg/kg lw in her milk, also suggesting that it had not yet successfully offloaded (see SText: case id UT529).

4. Discussion

Here we present the levels of exposure of PCBs, PBDEs, HCB and HCBD of 121 harbour porpoises of all maturity classes that were stranded along the Dutch coast (2008–2019), including nine foetuses. This study represents the most recent and comprehensive investigation into generational cycling of persistent organic pollutant in an abundantly stranded small cetacean in the southern North Sea. We included samples of infrequently accessible foetuses, placenta, umbilical cord and milk and combined this with data on causes of death, nutritional condition and parasitology from necropsies.

4.1. Uptake and elimination in maturity classes

Concentrations of organic contaminants in blubber samples of harbour porpoises differed between sex and maturity classes (Fig. 1, STab 5). These differences in contaminant concentrations can be explained by maternal transfer, specifically offloading of contaminant by mature females to their offspring, as well as differences in the uptake via diet (Jepson et al., 1999; Sørmo et al., 2003; Williams et al., 2020a).

In general, contaminant concentrations were lowest in foetuses. Foetuses obtain organic contaminants from their mother via transplacental transfer during gestation (Desforges et al., 2012). The transfer of contaminants is influenced by the chemical characteristics of the contaminants. PCB congeners with a low molecular weight can move easier across the placenta than larger congeners (Salata et al., 1995; Borrell and Aguilar, 2005). This was also observed in this study, with a predominant transfer of Tetra and Penta PCBs and Tetra PBDEs from the mother to the foetus. Duinker and Hillebrand (1979) estimated that 15% of the organochlorine contaminants in harbour porpoise adult females were transferred to their foetuses, with foetuses having an average weight of 17% of the mass of their mother. This is a higher percentage than in other (larger) odontocete species, in which adult females transferred an estimated 4–10% of total body burdens of organochlorine compounds to their foetus during gestation (Fukushima and Kawai, 1981, in: Yordy et al., 2010; Borrell et al., 1995).

Neonate harbour porpoises contained higher contaminant concentrations than foetuses. Concentrations of organic contaminants in neonates are a result of both transplacental transfer and lactational transfer. Transfer rates during lactation are considered higher than during transplacental transfer (Mongillo et al., 2016). Our results showed that milk resembled PCB and PBDE profiles of the blubber samples of the adult females. This means that after birth neonates receive a higher proportion of higher halogenated congeners (Hepta/Octa PCBs, Hexa PBDEs). Hence their profiles become more similar to those of older animals.

First born calves are thought to receive higher contaminant loads than subsequent calves (Cockcroft et al., 1989; Yordy et al., 2010; Lundin et al., 2016; Mongillo et al., 2016), which may result in up to four times higher concentrations in first-born calves, as was suggested for striped dolphins (*Stenella coeruleoalba*) (Fukushima and Kawai, 1981, in: Yordy et al., 2010), bottlenose dolphins (*Tursiops truncatus*) (Cockcroft et al., 1989) and long-finned pilot whales (*Globicephala melas*) (Borrell et al., 1995). Our results suggest the same, with adult females with a smaller total length having higher PCB levels in milk. A decrease in PCB levels in milk of adult females with a larger size can be explained with a high offloading in firstborn calves. A linear relation between total length and PCB/PBDE concentration in milk was therefore not expected.

Foetuses and neonates are more susceptible to toxic effects of POPs than older, more developed individuals. Effects of the contaminants can be exerted directly on the foetus and neonate, or indirectly via the mother and/or the placenta, or both (Rogers and Kavlock, 2001). Young individuals may also have a lower capability to metabolise and eliminate compounds (Weijs et al., 2010a). The high developmental rate of foetuses and neonates enhances the potential for toxicological effects. Williams et al. (2020a) concluded that calves were likely to be exposed to more neurotoxic PCB congeners (Tri-Penta PCBs) than adult harbour porpoises. These effects may eventually result in a lower survival of exposed individuals (Borrell and Aguilar, 2005).

Juvenile harbour porpoises contained slightly higher contaminant concentrations than neonates and these levels were similar for both sexes. In juveniles the diet shifts gradually from milk to prey, resulting in new sources for uptake of organic contaminants (Boon et al., 2002; Yordy et al., 2010). Juvenile and young adult odontocetes experience high growth rates during this life stage requesting higher metabolic rates and/or increased feeding rates, as was also observed in bottlenose dolphins (Yordy et al., 2010). Rapid growth may enhance the bioaccumulation of organic contaminants through increased intake of food. PCB congener profiles of juveniles were similar to those of the neonates with a slightly higher proportion of Hepta PCBs. Compared with adults, juveniles still had higher proportions of Tri to Penta PCBs, which was also observed in earlier studies in Dutch waters (Weijs et al., 2007, 2009).

The highest PCB and PBDE concentrations were found in adult male harbour porpoises. An average PCB accumulation rate of 1.1 mg/kg lw per year through dietary intake was estimated for male harbour porpoises based on levels in stranded individuals (Murphy et al., 2015). Adult males are only able to eliminate some of the congeners via metabolism and consequent elimination. Of the Sum-17PCB, PCB congeners 28, 105, 118, 156 were described to be (partly) metabolised by CYP1A1 in cetaceans (Boon et al., 1997a; Cullon et al., 2012). Both adult males and adult females showed concentrations of these congeners that were a factor 2–3 lower than in foetuses, neonates and juveniles. This further supports the hypothesis that younger age classes have a lower ability to eliminate compounds than adults. Adult males in the current study showed a higher proportion of Hexa, Hepta and Octa PCBs, compared with the other maturity classes, pointing at retainment of mainly highly persistent PCBs in adult males. In male bottlenose dolphins, the concentrations of persistent (non-degradable) PCBs increased with age (Yordy et al., 2010). Larger PBDE congeners have a much lower potential to be taken up by marine mammals through food web transfer than PCBs (De Boer et al., 1998; Boon et al., 1997b), which was supported with our findings that the dominant PBDE group in adult males was the Hexa PBDEs, whereas Hepta to Deca BDEs were hardly observed.

Adult female harbour porpoises had lower PCB, PBDE and HCB concentrations in their blubber compared with adult males. Female cetaceans can offload organic contaminants partly through metabolism (as do males), but particularly through transplacental transfer and lactation, resulting in a loss of contaminant body burden of the adult females of up to 60 to nearing 100% (Fukushima and Kawai, 1981, in: Yordy et al., 2010; Borrell et al., 1995; Wells et al., 2005; Murphy et al., 2015).

The amount of offloaded contaminant as well as the exposure rates during lifetime are influenced by the age of the adult and the number of calves given birth to, and therefore varies per individual (Mongillo et al., 2016). The highest amount of offloading is thought to occur during the first successful pregnancy, predominantly through lactation (Borrell et al., 1995). This corresponds to our results, which showed that increasing total length (as a proxy for age) was related to a decrease in the amount of contaminants that were offloaded. After lactation, contaminants can again be taken up through prey ingestion, as this was observed in bottlenose dolphins (Yordy et al., 2010), although concentrations as in earlier life stages (prior to lactating) will not be reached again (Murphy et al., 2015).

Age, previous pregnancies, nutritional status and month may have influenced contaminant and lipid levels in milk. PCB concentrations in milk were lower in adult females with a greater total length and presumably older age. Besides this, lipid % in milk of emaciated females was lower than those that had a better nutritional status. Therefore nutritional status of females presumably also strongly influenced how much contaminants were offloaded into the milk. Adult females with a poor nutritional condition had higher PCB concentrations in milk than those in a moderate or good nutritional condition, probably due to mobilisation of lipid stores and as such PCBs. Similar findings were reported for southern resident killer whales (*Orcinus orca*), where contaminant concentrations, which likely originated from endogenous lipid stores, were highest and had the greatest potential for toxicity in periods of low prey abundance (Lundin et al., 2016).

PCB and PBDE levels in milk were highest during early lactation (April–August) compared to late lactation, whereas lipid levels were highly variable and did not show a clear pattern between early and late lactation. Longitudinal studies on bottlenose dolphins showed that levels of organic compound releasement through milk were related to age, reproductive history and lipid content of the milk (Ridgway and Reddy, 1995; Yordy et al., 2010). In grey seals (*Halichoerus grypus*) PCB levels increased from early to late lactation. This was explained by a higher mobilisation of PCBs through changes in the maternal blubber composition as a result of lipid loss by the mother (Debier et al., 2003). However, both the lactation period and intensity between grey seals and harbour porpoises differ greatly. Grey seals are capital breeders with a short lactation period (18 days on average) in which they mobilise maternal blubber stores to produce fat rich (50–60% lipid) milk (Hall and Russell, 2018). Harbour porpoises have a lactation period of approximately 10 months and feed the entire time during lactation. Additional collection and analyses of milk samples of harbour porpoises will provide further insight into the variables influencing the contaminant levels in milk of harbour porpoises, despite the fact that it is challenging to acquire a sufficient number, volume and quality of milk samples in cetaceans.

4.2. Comparison to other studies

PCB concentrations in our study were in the same range to earlier reported PCB concentrations in harbour porpoises of the southern North Sea and UK waters (Pierce et al., 2008; Weijs et al., 2009; Mahfouz et al., 2014; Jepson et al., 2016; Williams et al., 2020b). However, variation between individuals is high and some maturity classes in earlier studies had higher average concentrations than animals in our study (Mahfouz et al., 2014; Weijs et al., 2009). Lower PCB concentrations can be found in harbour porpoises in the Black Sea (Weijs et al., 2010b) and southwest Greenland (Borrell et al., 2004), and higher concentrations in those from the Northwest Iberian Peninsula (Méndez-Fernandez et al., 2014).

PBDE concentrations in our study were similar to earlier reported PBDE concentrations in harbour porpoises inhabiting the southern North Sea, although concentrations in adults were slightly lower than what was reported before (Weijs et al., 2009; Pierce et al., 2008). Lower PBDE concentrations can be found in harbour porpoises of the Black Sea (Weijs et al., 2010b).

HCB concentrations in harbour porpoises of our study were lower than in earlier reported studies of the southern North Sea (Imazaki et al., 2015) and comparable to those reported from southwest Greenland (Borrell et al., 2004).

PCB concentrations in harbour porpoises were a factor 3–7 lower than in other larger toothed cetaceans of the Northeast Atlantic, such as the bottlenose dolphin, the striped dolphin and the killer whale (Andvik et al., 2021; Jepson et al., 2016). Population declines in bottlenose dolphins and killer whales in the Northeast Atlantic were thought to be predominantly driven by bioaccumulation of PCBs (Jepson et al., 2016). The higher PCB concentrations in these other odontocete species can be explained by their longer life expectancy, with a later age at sexual maturity, and the fact that they feed at higher trophic levels (Hall et al., 2006b; Jepson et al., 2016; Desforges et al., 2018).

PCB concentrations in harbour porpoises were in the same range as those in harbour seals (*Phoca vitulina*) in the North Sea, whereas PBDE concentrations were approximately a factor two higher in harbour porpoises than those in harbour seals (Weijs et al., 2009). Harbour porpoises contained a higher proportion of lower chlorinated PCBs than harbour seals. This was explained by the fact that harbour seals were thought to have a better capacity to metabolise PCBs than harbour porpoises (Boon et al., 1997a). This results in a higher bioaccumulation in harbour porpoises and could imply an increased risk for adverse health effects in this species (Weijs et al., 2009).

4.3. Potential health effects

4.3.1. Parasites

No significant correlation between the parasite loads and PCB concentrations in blubber was observed in our study. This is in contrast to a study conducted in the UK, where a significant, positive correlation between PCB levels and nematode burdens in stranded harbour porpoises was found, although the nature of the relationship was confounded by the porpoise's sex, age and cause of death (Bull et al., 2006). Also, individuals with the most severe nematode infestations did not have the highest PCB concentrations and therefore Bull et al. (2006) stated that while PCBs are important, they were clearly not the sole determinants of nematode burdens in harbour porpoises around the UK. Although scoring of parasite burden was done similarly to Bull et al. (2006), methodological differences could account for the different outcomes between the study by Bull et al. (2006) and ours. These authors used classification trees generated through recursive partitioning and fitted their models using binomial recursive partitioning, while we incorporated the parasite load data directly into the generalized linear modelling procedures. Additionally, more than twice as many cases were assessed in the UK compared to the current study. Bull et al. (2006) found the highest levels of Sum-25PCB to be associated with intermediate levels of both bronchiole and pulmonary nematodes and with high levels of nematodes found in the cardiac stomach, but they could not provide a mechanistic explanation. Additional empirical studies on the relationship between contaminant levels and host immunocompetence would improve the understanding of the harbour porpoise-parasite relationship and the factors that may influence this.

4.3.2. Causes of death

Harbour porpoises with high PCB concentrations died more often from an infectious disease and/or debilitations (like severe emaciation) than from an acute cause of death, such as bycatch or predation. However, a significant relationship between causes of death and PCB concentrations could not be confirmed in our analyses. The number of animals with PCB concentrations above the thresholds, especially the threshold of 41 mg/kg lw ($n = 6$), was low. However, with the odds ratio close to 0 (−0.004;0.055), a potential relationship between cause of death and PCB contaminants cannot completely be ruled out for this study. The interaction in the final model between sex and maturity class was explained by the difference in PCB concentrations between adult males and adult females due to offloading. Additionally the interaction between cause of death and nutritional condition was expected, as animals dying from acute causes were often in better nutritional condition than those dying from prolonged health problems.

In harbour porpoises from UK waters and the southern North Sea the risk of death from infectious disease was previously associated with increasing PCB exposure (Hall et al., 2006a; Mahfouz et al., 2014; Jepson et al., 2016). Hall et al. (2006a) reported that for each 1 mg/kg increase in blubber PCBs, the average increase in risk of infectious disease mortality was 2% and a doubling of risk occurred at approximately 45 mg/kg lipid. In UK waters, all female porpoises with PCB burdens above 30 mg/kg lw died from either infectious disease or other non-traumatic causes of death (Murphy et al., 2015). This was also found in our study; the two adult females with very high PCB concentrations in their blubber (39.7 and 76.2 mg/kg lw) had died due to an infectious disease. Increasing the sample size of animals is recommended to further test this hypothesis.

4.3.3. Reproduction

In mammals at large, the reported effects of endocrine-disrupting chemicals such as PCBs and DDTs on reproduction include reproductive tract anomalies, polycystic ovarian syndrome, ovarian failure, uterine fibroids, endometriosis, neoplasm and ectopic gestation, all of which may lead to infertility, spontaneous abortion, changes in age at sexual maturity, and lactational and ovulational failure (reviewed in: Diamanti-Kandarakis et al., 2009). For marine mammals and specifically cetaceans, the number of cases where organic compound exposure could be associated with reproductive failure is low (Murphy et al., 2018), but cases of foetal and/or neonatal mortality in harbour porpoises (Murphy et al., 2015) and cases of increased firstborn-calf mortality in bottlenose dolphins (Schwacke et al., 2002; Wells et al., 2005) have been described. It has been proposed that if PCB levels exceed 11 mg/kg lw in resting, sexually mature female harbour porpoises, individuals should be regarded as nulliparous, as infertility or reproductive failure is expected (Murphy et al., 2015). Four out of 38 adult females in our study had PCB concentrations exceeding this level. Ovaries were assessed in 20 out of the 38 adult females. Within this small sample size, we found clear signs of reproduction failure in one case (1/20), where PCB concentrations of 39.7 mg/kg lw in blubber were found in combination with a high number of ovarian corpora scars (15) and low age (7 years). No significant further relation between the number of ovarian corpora scars in all other adult females and Sum-17PCB or Sum-17PBDE concentration in their blubber could be detected. This strongly suggests that reproduction failure in the form of infertility (e.g. failure of ovulation, conception and implementation) does not occur at large. This is supported by studies undertaken on both harbour porpoises and common dolphins (*Delphinus delphis*), where exposure to PCBs did not inhibit ovulation, conception or implantation, but instead may have impacted foetal and new-born survival rates (Murphy et al., 2010, 2015, 2018).

In UK waters, resting harbour porpoise females were more likely to have higher PCB burdens than other maturity groups and, where data were available, these non-offloading females were shown to have previously been gravid, which also suggests foetal or new-born mortality (Murphy et al., 2015). As lower chlorinated and brominated compounds are more readily transferred, this means that foetus and neonates are exposed to a more neurotoxic mixture of these contaminants than observed in adults (Williams et al., 2020a). Although the direct and indirect mechanisms are yet to be established, one explanation could be that even low levels of POP exposure or difference in the mixture of POPs can have adverse health effects when exposure occurs at critical periods of development. It is conceivable that the threshold levels of negative effects on e.g. foetuses, neonates and juveniles in puberty are therefore lower than the current threshold levels and therefore re-evaluation in light of age and development stages may be necessary, as previously suggested for bottlenose dolphins (Hall et al., 2006b).

4.4. Management implications

PBDE concentrations and more recently also PCB concentrations have declined in UK waters, after concentrations reached an initial plateau, as a consequence of legislation of the production and disposal (Law et al., 2012; Law, 2014; Williams et al., 2020b). Over the past decade, mean blubber PCB concentrations fell below the proposed thresholds for toxic effects (Williams et al., 2020b). Trends for Dutch coastal waters cannot readily be determined based on the currently available data since sample size per year was too low for each maturity class. As trends vary over the region and measured PCB concentrations are still associated with increased rate of mortality due to infectious diseases, PCB mitigation is advised supplementary to earlier strict international regulations (Williams et al., 2020b). Open applications were reported to still release PCBs into the environment, and renewed mitigation measures may therefore provide further success to eliminate the release of these persistent contaminants into the environment (Stuart-Smith and Jepson, 2017).

Monitoring of PCBs and other relevant POPs in stranded harbour porpoises is an efficient tool to assess trends and potential effects of these contaminants in the marine environment, if proceeded for the long-term. Three types of assessment are recommended. Firstly, to determine maximum levels and trends in population, it is advised to focus on concentrations in blubber of adult males, as this maturity group contains the highest PCB and PBDE concentrations, reducing the bias of offloading which is apparent in the mature females. Important confounding factors such as decomposition and nutritional status should be taken into account as these affect the observed contaminant levels. Secondly, since the correlation between PCB and PBDE levels in blubber of the harbour porpoise showed strong correlations, measured

PCB concentrations can be used to predict PBDE levels in these individuals. It should however be noted that the observed range is rather broad. Therefore such a prediction should only be used as an indicative value. Thirdly, to obtain a better insight into population reproduction parameters, it is advised to retain a focus on the functions of the female reproductive system. A further assessment of routes of generational transfer of pollutants from adult females to their offspring as well potential health effects, especially in the developing calf, is needed to better understand to what level pollutants as PCBs and PBDEs may impact the health of the harbour porpoise population.

5. Conclusions

In this study, we assessed the exposure level of harbour porpoises from the southern North Sea to PCBs, PBDEs, HCB and HCBD. Besides analysing these contaminants in the blubber of stranded neonate, juvenile and adult porpoises, we were able to analyse tissues of foetuses, as well as milk, placenta and umbilical cord samples. These samples are rarely analysed in cetacean research and this novel approach therefore sheds light on exposure levels and generational transfer through different pathways. We found that the contaminants were transferred from females to their offspring via both the placenta and via lactation, with the latter being the most dominant transfer route. Lactation resulted in a high concentration of chlorinated and brominated contaminants in calves, at the start of each new generation.

We assessed the relation between PCB levels with health status of porpoises and showed that specifically adult males, together with half of the neonates and juveniles of both sexes, had PCB concentrations that exceeded the threshold level of negative effects. Porpoises with PCB levels above the highest threshold level of 41 mg/kg died more often of infectious disease and/or debilitation. Finally, nutritional stress caused mobilisation of the endogenous lipid stores leading to higher offloading through milk, leading to a greater potential for toxicity in calves of nutritionally stressed females. These results provide further evidence of the potential health effects of PCBs on the reproduction system of harbour porpoises of the southern North Sea with consequences for population viability.

CRediT authorship contribution statement

Martine J. van den Heuvel-Greve: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft, Funding acquisition. **Anneke M. van den Brink:** Visualization, Writing – review & editing. **Michiel J.J. Kotterman:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing. **Christiaan J.A.F. Kwadijk:** Investigation, Resources, Writing – review & editing. **Steve C.V. Geelhoed:** Writing – review & editing, Project administration, Funding acquisition. **Sinéad Murphy:** Investigation, Resources, Writing – review & editing. **Jan van den Broek:** Methodology, Data curation, Formal analysis. **Hans Heesterbeek:** Supervision, Writing – review & editing. **Andrea Gröne:** Supervision, Writing – review & editing. **Lonneke L. IJsseldijk:** Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Funding acquisition.

Declaration of competing interest

This manuscript is original work, has not been published previously, and is not under consideration for publication elsewhere. There are no copyright, financial or ethical issues with our work. The authors declare to have no competing interests.

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Appendix A. Supplementary data

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