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Concentrations and endocrine disruptive potential of phthalates in marine mammals from the Norwegian Arctic

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ABSTRACT

This study investigated concentrations of phthalates (diesters of phthalic acids) in blubber/adipose tissue of blue whales (*Balaenoptera musculus*), fin whales (*Balaenoptera physalus*), bowhead whales (*Balaena mysticetus*) and polar bears (*Ursus maritimus*) sampled in the Svalbard Archipelago (extending westward in the case of bowhead whales). Additionally, total concentrations (free and conjugated forms) of eight phthalate monoester metabolites were analysed in plasma of polar bears. Bis(2-ethylhexyl) phthalate (DEHP) was the only phthalate quantified among the 12 phthalates investigated. This compound was present in 6/7 fin whale samples, 4/7 blue whale samples, 2/5 bowhead whale samples and 1/12 polar bear samples. DEHP concentrations ranged from <20–398 ng/g wet weight. Phthalate metabolites, mono-*n*-butyl phthalate and monoisobutyl phthalate, were found in low concentrations (<1.2 ng/mL) in some of the polar bear samples. *In vitro* reporter gene assays were used to assess transcriptional activity of fin whale peroxisome proliferator-activated receptor gamma (PPARG), glucocorticoid receptor (GR) and the thyroid hormone receptor beta (THRB) by DEHP and diisononyl phthalate (DiNP). Due to the high degree of similarity of the ligand binding domain in the THRB and PPARG among whales, polar bears and humans, the transactivation results also apply for these species. DEHP showed both agonistic and antagonistic effects towards whale THRB at considerably higher concentrations than measured in the study animals; DiNP was a weak agonist of whale THRB. No significant agonistic or antagonistic effects were detected for DEHP or DiNP for whale PPARG, whereas DEHP and DiNP decreased basal luciferase activity mediated by whale GR at several test concentrations. In conclusion, DEHP was detected in the blubber of marine mammals from the Norwegian Arctic and it appears to have potential to modulate the transcriptional activity of whale THRB, but current DEHP concentrations do not modulate the function of the studied nuclear receptors in adipose tissue of blue whales, fin whales, bowhead whales or polar bears sampled from the Norwegian Arctic.

1. Introduction

Phthalates (diesters of phthalic acids) are widely used as plasticizers to impart flexibility to plastics (Hansen et al., 2013; Wilkes et al., 2005). The global production of phthalates was estimated to be 5.5 million tonnes per year in 2018 (Holland, 2018). Bis(2-ethylhexyl) phthalate (DEHP) is a widely used phthalate, but due to concern over its toxicity and subsequent regulation (Eichler et al., 2019; Katsikantami et al., 2016; Ventrice et al., 2013), the use and occurrence of DEHP in Europe and North America has decreased in recent years (ECPI, 2019; Nagorka and Koschorreck, 2020). DEHP, benzyl butyl phthalate (BBP), di-*n*-butyl phthalate (DnBP) and diisobutyl phthalate (DiBP) are listed under the European Chemical Agency's Candidate List of substances of very high concern for Authorisation because it is toxic to reproduction and is an endocrine disruptor (https://echa.europa.eu). In the USA, several phthalates are currently undergoing risk evaluation for the Toxic Substances Control Act (www.epa.gov).

DEHP has been replaced for most applications by another high molecular weight phthalate, DiNP (ExxonMobil Petroleum and Chemical B.V.B.A 2014), which is currently (2020) manufactured in or imported into the European Economic Area in large quantities (100 000–1000 000 tonnes annually; https://echa.europa.eu). Consequently, DiNP has

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become the dominant phthalate, sometimes along with DEHP, in particulate matter from German rivers, and effluent and sludge from waste water treatment plants and sediments from the Nordic countries (Nagorka and Koschorreck, 2020; Remberger et al., 2013). The shift from DEHP to DiNP is seen in human biomonitoring data from the USA (Reyes and Price, 2018). DEHP continues to be produced in parts of the world (ECPI, 2019) and is, together with other phthalates, ubiquitous in the environment (Gao and Wen, 2016; Net et al., 2015).

Phthalates are present even in remote parts of the globe such as the Arctic. A significant deposition of phthalates from Arctic air to seawater indicates that atmospheric transport is an important pathway for long-range transport of phthalates (Xie et al., 2007). Additionally, (micro) plastic carried by ocean and air currents (Bergmann et al., 2019; Halsband and Herzke, 2019) may act as a transport vector for phthalates. Plastics are found all over in the Arctic including in ocean surface water (Lusher et al., 2015), deep sea sediment (Bergmann et al., 2017b), sea ice (Kanhai et al., 2020) and along shorelines (Bergmann et al., 2017a). Microplastics have also been found in Arctic whales and seabirds (Amelineau et al., 2016; Baak et al., 2020; Moore et al., 2020). Phthalates can migrate (leach) from plastic into the environment or directly into an organism because they are not chemically bound to polymer matrixes (Hahladakis et al., 2018 and references therein; Paluselli et al., 2019). Detection of phthalates in filter-feeding whales from the Mediterranean Sea and Sea of Cortez have been related to high concentrations of microplastics in their feeding areas of whales in these regions (Fossi et al., 2016). It is thus importance to address the current lack of knowledge regarding the presence of plastic additives, such as phthalates, in marine mammals from the Arctic.

The presence of phthalates in marine biota is of high concern. The dominant phthalates DEHP, DiNP and DiDP have high octanol-water and octanol-air partition coefficients (Cousins and Mackay, 2000), which make them prone to bioaccumulation in lipid-rich tissues and biomagnification in marine food webs (Kelly et al., 2007). However, phthalate diesters can be biotransformed to more hydrophilic monoester metabolites in mammals (Hart et al., 2018; Rian et al., 2020; Silva et al., 2003). Studies on experimental animals, supported by clinical human studies, indicate that phthalates can impair development, reproduction, metabolism and cause neurological issues in addition to being carcinogenic (Benjamin et al., 2017). Phthalates act as hormone sensitizers and nuclear receptors, and other transcription factors related to the endocrine system are expected to be key mechanisms of phthalate toxicity (Baken et al., 2019; Benjamin et al., 2017). These include peroxisome proliferator-activated receptor gamma (PPARG/NR1C3), glucocorticoid receptor (GR/NR3C1) and the thyroid hormone receptor beta (THRB/NR1A2), which are transcription factors that regulate endocrine systems. PPARG is the “master regulator” of lipid metabolism in adipose tissue (Lefterova et al., 2014), whereas GR is involved in control of energy metabolism and suppression of inflammation among other functions (Vegiopoulos and Herzig, 2007). Thyroid hormones that act through THR regulate development, growth and metabolism (Mullur et al., 2014). To get insights into the endocrine disruptive potential of contaminants in free-living marine mammals, species-specific *in vitro* methods have recently been developed to study modulation of nuclear receptors by environmental contaminants (Lille-Langøy et al., 2015; Lühmann et al., 2020; Routti et al., 2016).

To provide information about exposure of phthalates in marine mammals from the Norwegian Arctic, phthalate diester concentrations were measured in blubber/adipose tissue of blue whales (*Balaenoptera musculus*), fin whales (*Balaenoptera physalus*), bowhead whales (*Balaena mysticetus*) and polar bears (*Ursus maritimus*) sampled from the Barents and Greenland seas. Additionally, plasma samples from polar bears were analysed for total concentrations of phthalate monoester metabolites including their free and glucuronated forms. Blue whales and fin whales used in this study are part of the Northeast Atlantic population that reside around Svalbard during summer, whereas bowhead whales and polar bears are endemic, full-time residents in the Arctic. To get insights about endocrine disruptive potential of phthalates in these species, *in vitro* reporter gene assays were used to assess transcriptional activity of PPARG, GR and THRB by DEHP and DiNP.

2. Material & methods

2.1. Solvents and standards

Methanol for liquid chromatography (LiChrosolv®) and acetone, cyclohexane, n-hexane for gas chromatography (SupraSolv®) and formic acid were sourced from Merck. Acetonitrile (LCMS grade) was purchased from Rathburn Chemicals Ltd (Walkerburn, Scotland). Deuterated (d4) DEHP purchased from Chiron AS (Trondheim, Norway), and d4-diethyl phthalate (DEP), d4-diisobutyl phthalate (DiBP), d4-dibenzyl phthalte (DBzP), d4-di-*n*-hexyl phthlate (DnHxP) and d4-dioctyl phthalate (DnOP) purchased from Accustandard Inc (CT, USA), were used as internal standards, whilst d4-dipropyl phthalate (DPP) (Accustandard Inc) was used as a recovery standard for phthalate analyses. Internal standards for phthalate metabolite analyses, d4-monoisobutyl phthalate (MiBP), d4-monobenzyl phthalate (MBzP) and d4-mono-(2-ethylhexyl) phthalate (MEHP) were acquired from TRC (Toronto, Canada) and d4-mono-ethyl phthalate (MEP) was sourced from Chiron AS. A recovery standard, d4-mono-*n*-octyl phthalate was sourced from TRC. Native phthalate standards, DEP, DiBP, DnBP, bis(4-methyl-2-pentyl) phthalate (DMPP), DnHxP, DEHP, dicyclohexyl phthalate (DCHP), DnOP, di-*n*-nonyl phthalate (DnNP), DiNP and DiDP were sourced from Accustandard As and used to create the calibration curve. Native phthalate metabolite standards, MEP, mono-*n*-butyl phthalate (MnBP), MBzP, MEHP, mono(2-ethyl-5-oxohexyl) phthalate (MEOHP) were acquired from Chiron As and MiBP and mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) were purchased from TRC.

2.2. Cleaning procedure

Phthalates frequently occur in indoor environments and are readily found in dust and indoor air, so special precautions were taken to keep glassware, samples and lab equipment clean. Deionized water (18.2 MΩ) was prepared using a Milli-Q Advantage water purification system and run through an LC-Pak polisher (Merck Millipore, Bedford, MA, USA) to remove background contaminants. All laboratory glassware was washed in a Miele laboratory washing machine using purified water and Extran detergent (Merck Millipore) and then dried. Glassware used in the field and laboratory were rinsed using cyclohexane and acetone. The glassware and aluminum foil used were heated in an oven at 450 °C for 8 h the day before use or prior to the field season. All glassware used in laboratory was also washed with acetonitrile immediately before use. Caps for glass vials used in the field were cleaned using an ultrasonic bath (10 min) and rinsed with acetone prior the field season. Biopsy darts for whale sampling, tweezers and scissors were washed with boiling water, rinsed with acetone with a glass pipette and packed individually in aluminium foil.

For phthalate diester analyses, all preparation of standards and samples, as well as extraction and cleanup, were done in a laminar flow cabinet (Chemcap cabinet from Bigneat Ltd, UK) with an HEPA and carbon filter on both the inlet and exhaust. Ultrasonication (USC 1200 THD, VWR International LLC) and evaporation using a Mivac Quattro centrifugal vacuum concentrator (Genevac Ltd, Ipswich, Suffolk, UK) was carried out outside the laminar flow cabinet but always for limited times, and samples were always covered with aluminum foil and capped when they were moved from place to place in order to avoid contamination.

2.3. Field sampling

Seven blue whales and six fin whales were biopsied from small boats using pre-cleaned custom-made biopsy darts made of stainless steel (10

cm long) shot from a crossbow in May to September in 2017–2018 off the west coast of the Svalbard Archipelago (Fig. 1). The dart was attached to a string secured to the crossbow that enabled recovery of the sample. Five bowhead whales were biopsied west of Svalbard (Fig. 1) during June 2017 in a similar manner, but from a helicopter (Eurocopter AS350 Ecureuil), using a shorter (4 cm), similarly made stainless steel biopsy dart that bounces off the whale and floats at the surface where it is recovered using a small hoop net from the aircraft. Sex of the whales was identified by molecular sexing (Berube and Palsboll, 1996). Adult polar bears were captured in Svalbard (Fig. 1) in March-April 2017. The bears were immobilized by remote injection of tiletamine hydrochloride and zolazepam hydrochloride (Zoletil Forte Vet ®; Virbac, France) from a helicopter (Eurocopter AS350 Ecureuil). Age estimation of polar bears was done from vestigial premolar tooth (Christensen-Dalsgaard et al., 2010), except for bears captured during previous field seasons as juveniles that were hence of known age. Twelve adipose tissue samples were collected using an 8 mm sterile disposable biopsy punch (Fray Products, Buffalo, NY, USA or Miltex, Japan), taken approximately 15 cm lateral to the base of the tail. The upper layer of whale blubber, approximately 1–5 cm from the skin, and whole polar bear biopsies, were packed in glass vials and aluminium foil was placed inside of a polypropylene top cap, with septum lined with polytetrafluoroethylene (PTFE) foam urethane (National Scientific, Austin, Texas, USA). Blood samples were collected from the femoral vein of 13 polar bears using heparinized tubes. The tubes were centrifuged within 10 h and plasma was moved into sterile cryovials made of polypropylene. The samples were stored at −20 °C until analyses. The sampling was approved by the National Animal Research Authority of Norway and the Governor of Svalbard.

2.4. Sample preparation for phthalate diester analyses

A frozen biopsy of 0.2 g was cut with scissors into a 15 mL centrifuge glass tube and mixed with 2 g of Na_2SO_4 (dried at 600 °C for 8 h) and left overnight at −20 °C covered with aluminum foil and a PTFE lid. One hundred ng of d4-DEHP was added to each sample as an internal standard and let stand for 30 min. Samples were extracted by ultrasonication at 35 °C, 45 kHz for 10 min two times with 5 mL of (1:1 v/v) acetone/n-hexane and then transferred to another 15 mL centrifuge glass tube and evaporated to near dryness in a vacuum concentrator, after which 100 μL of isooctane was added.

Cleanup of the samples was done using solid phase extraction using a dual layer 105 mm × 17 mm glass cartridge with an Elufix (LCTech GmbH, Obertaufkirchen, Germany) and a 12 position Supelco solid-phase extraction (SPE) manifold (Merck KGaA, Darmstadt, Germany). The cartridge was packed with 1.25 g mixture of a zirconia coated silica and octadecyl (C18) bonded silica in the bottom and separated with a frit and 1.25 g activated Florisil was added on top and one more frit was placed on top of the material (Merck KGaA, Darmstadt, Germany). Subsequently, the column was washed with 10 mL of acetonitrile using gravity flow and then it was vacuum dried for 10 min at −15 mmHg, using a portable membrane vacuum unit. The sample was added to the cartridge and a few drops of additional isooctane was used to rinse the 15 mL glass tube and an additional rinsing was conducted using 0.5 mL of acetonitrile, both rinses were transferred to the cartridge.

The sample was eluted using 3 × 5 mL of acetonitrile, and 13 mL of the eluate was collected in a 15 mL centrifuge glass. The samples were evaporated to 200 μL in a vacuum concentrator and transferred using a small amount of acetonitrile to 1.5 mL analytical glass vials with a 300 μL glass insert and 10 ng of syringe standard (d4-dioctyl phthalate and d4-dipropyl phthalate) and 50 μL of 0.2% formic acid in cleaned deionized water were added to the sample.

2.5. Sample preparation for phthalate metabolite analyses

Deconjugation and extraction of phthalate monoester metabolites were conducted following the methods outlines in Jeong et al. (2011), with a few modifications. Half mL of plasma, 100 μL of 1 M ammonium acetate, 20 μL of internal standard mixture (d4-MEP, d4- MiBP, d4-MBzP

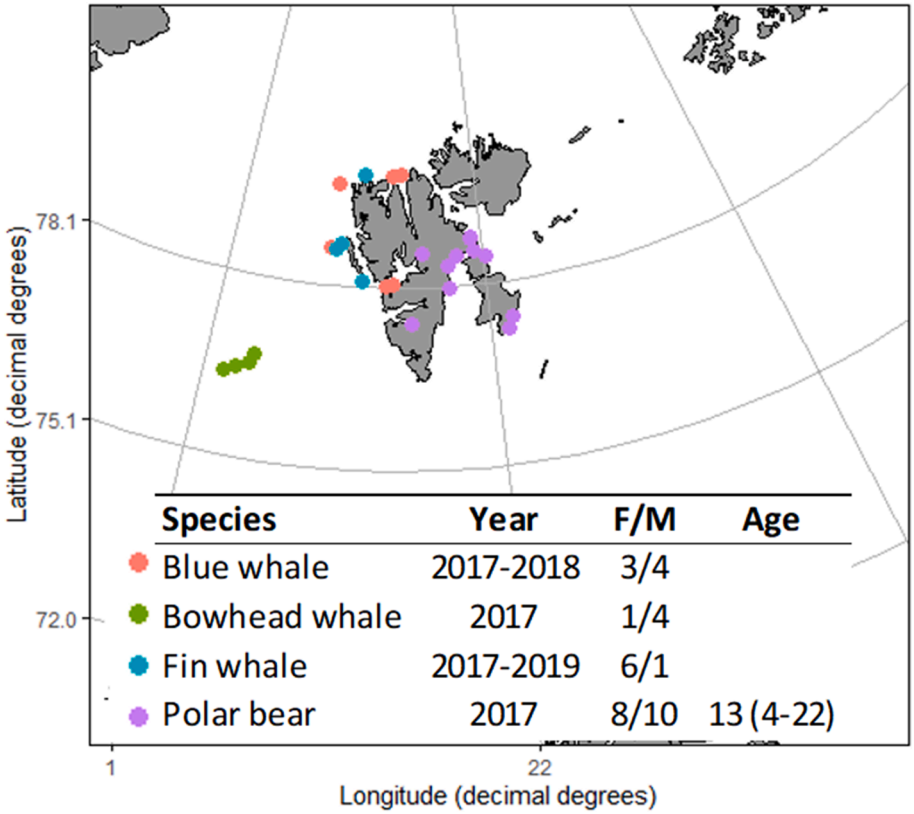

Fig. 1. Sampling information of blue whales, bowhead whales, fin whales and polar bears.

and d4-MEHP) and 10 μL β-glucuronidase (BGTurbo > 200 000 units/mL from Kurabiotech, Chile) were mixed gently in a 2 mL glass screw cap vial and incubated at 37 °C for 2 h. The mixture was diluted with 1 mL 1% formic acid in acetonitrile and mixed with vortex, sonicated for 10 min and centrifuged for 10 min. A 1 mL ion exchange SPE Oasis MAX column (Waters Inc, Milford, MA, USA) was washed with 1 mL methanol and conditioned using 20% methanol in cleaned and deionized Milli-Q water. One mL of 5% ammonium hydroxide solution and the supernatant was added on top of the SPE column and thoroughly mixed with a pasteur pipette before elution on the 12 position SPE manifold, which was connected to a membrane vacuum pump. The column was rinsed with 1 mL 5% ammonium hydroxide solution and 1 mL methanol which were discarded, and the analytes were eluted using 1 mL 2% formic acid in methanol into a 2 mL glass vial. Samples were evaporated under a gentle flow of N2 to 100 μL, diluted with 300 μL Milli-Q water and filtered with a Mini-UniPrep 0.45 μm filter.

2.6. Analysis

Analyses were run on a triple quadrupole mass spectrometer (TSQ Vantage, Thermo Fisher Scientific Inc, Waltham, MA, USA) with a Waters XBridge C18 2.1 mm × 50 mm, 5 μm particle trapping column between the pump and injector to prevent background contamination in the Ultra Performance Liquid Chromatography (UPLC) system. For the analyses of phthalates diesters, the UPLC was fitted with an Acquity UPLC BEH Phenyl column 1.7 μm 2.1 × 100 mm with a 5 mm guard column (Waters Inc), which were held at 40 °C in a column oven, using an eluent of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B) at a flow rate, starting at 0.3 mL/min and increasing to 0.4 mL/min at 100% B. Ionization was achieved using positive electrospray ionization at 5000 V, heated to 380 °C with nitrogen as a sheath gas. Multiple reaction monitoring of the $M + H^+$ was achieved using Argon as the collision gas. Phthalate metabolites were analyzed with following modifications: the UPLC was fitted with a Cortecs solid core C18 column 2.7 μm 3.0 × 100 mm (Waters Inc) and negative ionization was achieved at 2500 V and 310 °C.

2.7. Quantification and quality assurance

Native standards were used to prepare a five point calibration curve from 50 to 1000 ng/mL for phthalate quantification and a seven point calibration curve from 1 to 500 ng/mL for phthalate metabolite quantification. Three procedural blanks were processed for each batch of samples. Limits of detection (LODs) and quantification (LOQ) for phthalates (Table S1) were calculated as the average concentration of phthalate in the procedural blanks (consisting of 2 g Na_2SO_4 for phthalates and Milli-Q water for metabolites) plus three and ten times the standard deviation, respectively. Phthalate concentrations in the samples were thus corrected for the presence of DEHP, DiNP, DiDP and phthalate metabolites in the procedural blanks. Field blanks were prepared consisting of 0.25 g of seal blubber sample in cleaned glass vials with cleaned aluminum foil and screw caps. One field blank was stored at −20 °C in a freezer in the sample processing laboratory as a reference and was processed at the same time as samples and field blanks. The other three field blanks were open in the helicopter/boat for a few minutes. Concentrations of phthalates were similar between the reference samples and field blanks. Recoveries for internal phthalate standards (d4-DEP, d4-DiBP, d4-DBzP, d4-DnHxP and d4-DEHP) in seal blubber, ranged from 41 to 97%. Recoveries for internal phthalate metabolite standards (d4-MEP, d4-MBzP, d4-MiBP, d4-MEHP) in human and polar bear plasma were between 32 and 94%. Analyses of four samples of Standard Reference Material 1957 (Organics Contaminants in Non-Fortified Human Serum, supplied from NIST, Gaithersburg, MD, USA) showed that the analytical precision of the phthalate metabolites varied between 5 and 7%.

2.8. Luciferase reporter gene assays

The agonistic and antagonistic effects of DEHP and DiNP on the receptor plasmids encoding for fin whale ligand binding domains (LBD) of PPARG, GR and THRB (henceforth referred to as wPPARG, wGR and wTHRB) were tested by luciferase gene reporter assays as described and validated in Lühmann et al. (2020) and Routti et al. (2016). Briefly, cells from the simian cell line COS7 were transiently transfected with the reporter plasmid tk(MH100)x4-luciferase, control plasmid pCMV-*β*-galactosidase, and either of the receptor plasmids pCMX-Gal4-wPPARG, pCMX-Gal4-wGR or pCMX-Gal4-wTHRB coding for fusion proteins of the yeast GAL4 DNA binding domain and the LBD of the corresponding receptor. COS7 cells were transfected using TransIT-LTI transfection reagent by Mirus (Madison, Wisconsin) at a mass ratio of 1:10:10. The control plasmid functioned as an internal standard for general cell viability and the efficiency of transfection. Twenty-four hours after transfection, the cells were exposed to test compounds for 24 h. Luciferase and β-galactosidase activities were assayed in cell lysates as changes in luminescence and absorbance, respectively. Functionality of the agonistic assays were tested by exposure to known ligands: rosiglitazone for PPARG (Lehmann et al., 1995), dexamethasone for GR (Tsurufuji et al., 1979) and T3 for THRB (Gross and Pitt-Rivers, 1953). Exposure concentrations for agonistic effects ranged from 8 nM to 50 μM. Antagonistic effects of test compounds were tested in the presence of a known ligand and the functionality of the assay was established by exposure to the known PPARG antagonist GW9662, the known GR antagonist mifepristone (RU486) and the known THRB antagonists bisphenol A (Cadepond et al., 1997; Leesnitzer et al., 2002; Moriyama et al., 2002; Zhang et al., 2015). For antagonistic effects, the tested concentration range was 40 nM to 50 μM. In each independent experiment, a solvent control and a positive control were included to confirm the functionality of the assay. At least three independent experiments, with three replicates, were performed per exposure scenario. All chemicals were sourced from Sigma Aldrich (St. Louis, Missouri).

2.9. Cytotoxicity

Membrane integrity and metabolic activity of COS7 cells was measured following the method described in Schreer et al. (2005). The dye resazurin was used to test for metabolic activity and 5-carboxyfluorescein diacetate (CFDA-AM) was used for to determine membrane integrity. The positive control Triton X-100, which is known to be cytotoxic for mammalian cells, was used (ranging from 0.00016% to 1% (v/v)) for the cytotoxicity assays. A minimum of three independent experiments, with three replicates each, were performed per phthalate.

2.10. Statistical analysis

The datasets analyzed during the current study are available in the Norwegian Polar Data Centre repository (Routti et al., 2021). The software R, version 4.0.2, was used for statistical analyses (R Core Team, 2020). To describe phthalate concentrations in different species, the R package *NADA* version 1.6–1 (Lee, 2017) was used to deal with data below the limit detection (non-detects). For data sets with <50% of non-detects, median, mean and standard deviation were calculated using the robust Regression on Order Statistics (ROS) method, as recommended by Helsel (2011). If detection ranges were lower, range was presented. The R package *EnvStats* version 2.3.1 was used to create probability plots for censored data (Millard et al., 2018).

For the luciferase reporter gene assays, β-galactosidase enzyme activity, resulting from the constitutive expression of the control plasmid, was used to correct luciferase activity for differences in transfection efficiencies between wells. The fold change in luciferase activity in exposed cells over solvent controls was the measured response for agonist experiments. For antagonistic experiments, the fold change was presented as luciferase activity in exposed cells over luciferase activity

Table 1
Concentrations (ng/g wet weight) of phthalates in blubber of fin whales, blue whales and bowhead whales and adipose tissue of polar bears. The numbers in parentheses show the number of carbons in the straight alkyl side-chain backbones. Number of samples (n) is given for the different batches with different detection limits. For detected compounds median[a] concentrations, range and number of samples above the limit of detection are given.

Compound	Abbr.	CAS	Blue whale (n = 7)	Fin whale (n = 6/1)	Bowhead whale (n = 5)	Polar bear (n = 6/6)
Diethyl phthalate (2)	DEP	84-66-2	<37.5	<37.5/<20	<37.5	<37.5/<20
Diisobutyl phthalate (4)	DiBP	84-69-5	<17	<17/<*18.1*[b]	<17	<17/<7.6
Benzyl butyl phthalate (4, aromatic ring)	BBP	85-68-7	<10	<10/<5.5	<10	<10/<5.5
Di-*n*-butyl phthalate (4)	DnBP	84-74-2	<17	<17/<5.5	<17	<17/<5.5
Dicyclohexyl phthalate (aromatic ring)	DCHP	84-61-7	<10	<10/<5.5	<10	<10/<5.5
Bis(4-methyl-2-pentyl) phthalate (4)	DMPP	84-63-9	<10	<10/<5.5	<10	<10/<5.5
Di-*n*-hexyl phthalate (6)	DnHxP	84-75-3	<10	<10/<5.5	<10	<10/<5.5
Bis(2-ethylhexyl) phthalate (6)	DEHP	117-81-7	20 (<12–101) 4/7	42 (<12–300) 6/7	n.a. (<12–398) 2/5	n.a. (<12–54) 1/12
Di-*n*-octyl phthalate (8)	DnOP	117-84-0	<10	<10/<5.5	<10	<10/<5.5
Diisononyl phthalate (8)	DiNP	28553-12-0	<100	<100/<75	<100	<100/<75
Di-*n*-nonyl phthalate (9)	DnNP	84-76-4	<10	<10/<5.5	<10	<10/<5.5
Diisodecyl phthalate (9)	DiDP	26761-40-0	<100	<100/<75	<100	<100/<75

[a] Median was calculated using robust Regression on Order Statistics method for species with >50% samples above the limit of detection.
[b] Detected but the value is below the limit of quantification (<18.1).

in cells exposed to the known agonist. Z values were used to detect and remove outliers, applying the z value of 1.96 criterion (Cousineau and Chartier, 2010). Linear mixed models were run using the R package *lme4* version 1.1-10 (Bates et al., 2015) to investigate whether DEHP and DiNP modulated the transcriptional activity of wTHRB, wPPARG and wGR, or the membrane integrity and metabolic activity of COS7 cells. The concentration was included as the fixed factor and the experiment number was used as the random factor to account for the variation between experiments. To verify that model assumptions (homogeneity, normality and independency, Zuur et al., 2010) were met, diagnostic plots were created, using the R package *DAAG* version 1.22 (Maindonald and Braun, 2015).

3. Results and discussion

3.1. Concentrations of phthalates and their metabolites

DEHP was the only phthalate that was quantified in the marine mammal blubber/adipose tissue analysed (Table 1). DEHP was detected in 6/7 fin whale samples, 4/7 blue whale samples, 2/5 bowhead whale samples and in 1/12 polar bear samples (Table 1). DEHP concentrations varied from <12 to 398 ng/g wet weight (ww); the highest value occurred in a bowhead whale sample. DEHP was also the predominant phthalate detected in blubber from four cetacean species (n = 1–3) and surface planktonic samples from the Mediterranean Sea (Baini et al., 2017). Concentrations of DEHP were much higher (ranging up to 26,000 ng/g dry weight - corresponding to 23,000 ng/g in wet weight (Kvadsheim et al., 1996)), in the Mediterranean Sea samples. DEHP was also the most abundant phthalate detected in marine mammal liver samples from Greenland (Vorkamp et al., 2004), where wet weight concentrations were similar to those reported in this study. However, it should be noted that DEHP concentrations in the Greenlandic samples were not corrected for relatively high blank contamination (>25 ng/mL). The presence of DEHP metabolites, MEOHP and MEHHP, in liver samples of harbour porpoises (*Phocoena phocoena*) from the Norwegian coastal waters and MEHP in urine of common bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, USA, suggest that other cetaceans are also exposed to DEHP (Hart et al., 2018; Rian et al., 2020). In the harbour porpoises from the Norwegian Coast, MEOHP and MEHHP were detected in 17 and 27% of the samples (n = 100), respectively, ranging from <0.17 to 5.9 ng/g ww.

Phthalate metabolites, MnBP and MiBP, were detected at low concentrations (≤1.2 ng/mL; all detected values > LOQ) in 1/13 and 3/13 polar bear plasma samples, respectively (Table 2). These were also among the most frequently detected metabolites in the harbour porpoises from the Norwegian Coast (Rian et al., 2020). MnBP and MiBP are likely metabolites of DnBP and DiBP, respectively. The low detection rate of phthalates and their metabolites in polar bears suggests that phthalates are present or accumulate to a lesser extent in the polar bear food web, or that polar bears excrete them rapidly. DiBP was detected in one fin whale sample at a concentration between the LOD and LOQ (7.6–18.1 ng/g ww) (Table 1). However it should be noted that the LOD for DiBP was as high as 7.6–17 ng/g ww and the presence of other phthalates should not be neglected in Arctic marine mammals because the LODs were in fact relatively high for the remaining phthalates (5.5–10 ng/g ww for BBP, DCHP, DMPP, DHxP, DnOP and DnNP, 5.5–17 ng/g ww for DnBP; 20–37.5 ng/g ww for DEP and 75–100 ng/g ww for DiNP and DiDP).

The high LODs set for phthalates, compared to LODs for e.g. legacy POPs, is because measured LODs include background contamination in blank samples. The ubiquitous presence of phthalates in the environment make it necessary to account for background values in order to

Table 2
Concentrations (ng/g wet weight) of phthalate metabolites in plasma of 13 adult polar bears. For detected compounds concentration range and number of samples above the limit of detection (LOD) are given.

Compound	Abbr.	CAS	Concentration	>LOD
Monomethyl phthalate	MMP	4376-18-5	<5.8	
Monoethyl phthalate	MEP	2306-33-4	<0.8	
Mono(2-ethyl-5-oxohexyl) phthalate	MEOHP	40321-98-0	<0.1	
Monoisobutyl phthalate	MiBP	30833-53-5	<0.2–1.2	3/13
Mono-*n*-butyl phthalate	MnBP	131-70-4	<0.2–0.63	1/13
Monobenzyl phthalate	MBzB	2528-16-7	<0.1	
Mono(2-ethyl-5-hydroxyhexyl)	MEHHP	40321-99-1	<0.1	
Mono(2-ethyl-1-hexyl) phthalate	MEHP	4376-20-9	<0.8	

interpret actual biotic contamination values. The blank contamination in this study was (for example 7.1 ng/g wet weight for DEHP) low compared to previously reported blank contamination values (Ikonomou et al., 2012). During the method development and analyses in the current study, a lot of effort was expended on minimizing background contamination by pre-cleaning all equipment, performing the extraction in a laminar flow cabinet where both the inlet and exhaust air was filtered and testing the presence of phthalates in solvents from different providers and standards. The samples were also taken with pre-cleaned equipment, and analyses of field blanks confirmed that the samples were not contaminated during the field sampling.

The source of DEHPs in the marine mammals from Svalbard are unknown. Although North Atlantic blue whales and most fin whales migrate to warmer waters for the winter months, they are likely exposed to phthalates in the Arctic, where they do much of their annual foraging effort (Lydersen et al., 2020; Pérez-Jorge et al., 2020). Analyses of sea water and air from the Norwegian Sea through into the Arctic indicate that DEHP is the predominant phthalate in aqueous dissolved phase and atmospheric particle phase from the North Sea to the high Arctic (Xie et al., 2007). More recent (2018) analyses of phthalate concentrations in air also indicated that DEHP, along with DiBP and DEP, were the most abundant phthalates in air samples from the Zeppelin Observatory, Ny Ålesund, Svalbard (Bohlin-Nizzetto et al., 2019). Based on estimation of net air/sea exchange fluxes, 30 and 190 tonnes/year of DEHP are deposited from the air to the Greenland Sea and Arctic Ocean, respectively, which suggests that atmospheric transport and deposition is a significant source of phthalates in the Arctic (Xie et al., 2007). Phthalates deposited into seawater are taken up by plankton, but both field and modelling studies suggest that DEHP and other phthalates do not biomagnify in aquatic food webs (Kim et al., 2016a; Mackintosh et al., 2004). Our results support this in that blue whales and fin whales that ingest large amounts of pelagic plankton, mainly krill (Gavrilchuk et al., 2014), had considerably higher concentrations of phthalates than polar bears (Table 1) that feed on the top of the Arctic marine food web (Derocher et al., 2002). Furthermore, DEHP metabolites, MEHP, MEHHP, MEOHP, were not detected in plasma of polar bears (Table 2). Trophic dilution of both phthalate diesters and their corresponding monoester metabolites (e.g. MEHP) have been shown in aquatic food webs up to fish, which suggests further metabolic transformation likely occurs in higher trophic level species (Hu et al., 2016).

Ingestion of plastic litter has been suggested to be a source for phthalate exposure in baleen whales (Baini et al., 2017; Fossi et al., 2014; Fossi et al., 2016). However, DEHP is likely found only at moderate concentrations in plastic from the upper layer of seawater, where the studied baleen whales feed (Croll et al., 2001; Nielsen et al., 2015). DEHP is mainly used in PVC (a typical concentration about 30% of weight (Hansen et al., 2013)), which is denser than seawater and thus subject to sinking in the water column (Halsband and Herzke, 2019; Malcolm Richard et al., 2011). Only 3–12% of microplastic particles in surface waters close to Svalbard and East Greenland were PVC (Amélineau et al., 2016; Lusher et al., 2015; Morgana et al., 2018). However, experimental studies have shown that phthalates can leach from polyethylene (PE), polypropylene (PP), and polystyrene (PS) (Hahladakis et al., 2018; Paluselli et al., 2019) demonstrating their use in other, positively buoyant, polymers (Malcolm Richard et al., 2011) might release them into surface waters. To what extent sorbed DEHP transfers from microplastic to whales is currently not known. A modelling exercise indicated that PVC ingestion was a negligible source of DEHP compared to intake from food and water in a benthic invertebrate, fish and seabird (Bakir et al., 2016). Indeed, a recent study did not detect any phthalates in the preen oil of northern fulmars (*Fulmarus glacialis*), which had ingested plastics (Provencher et al., 2020). Furthermore, leaching of DEHP from plastic to seawater is likely negligible due to DEHP's hydrophobicity and high partitioning coefficient (Paluselli et al., 2019).

DEHP concentrations ranged from <12 to 101 and 138 ng/g wet weight in blue whales and fin whales, respectively. Converted to lipid weight concentrations using previously reported lipid percentage in the same biopsies (Tartu et al., 2020; Routti et al., 2019), DEHP concentrations ranged from <20 to 237 and 394 ng/g lipid weigh in blue whales and fin whales, respectively. Comparison of DEHP concentrations in blue and fin whales to recently reported POP concentrations in the same individuals (Tartu et al., 2020; Routti et al., 2019) showed that DEHP concentrations overlapped with the concentration ranges of ΣCHLs and ΣPCBs, while ΣDDT concentrations were slightly higher, particularly in fin whales, and ΣPBDE concentrations were lower (Fig. 2). The DEHP results found in this study are not surprising when compared to air data from the Svalbard region. In air samples from Svalbard, phthalates were the predominant organic contaminants, with approximately 100 times higher concentrations than PCB concentrations (Bohlin-Nizzetto et al., 2019).

3.2. Endocrine disruptive potential of phthalates

Transactivation results for wTHRB, wPPARG and wGR reported herein are valid for several mammalian species. The LBDs of wPPARG and wTHRB are identical to the corresponding LBD of blue whales, killer whales (*Orcinus orca*), white whales (*Delphinapterus leucas*), polar bears and humans - apart from the last 15 amino acids that are lacking for the LBD of polar bear THRB. Fin whale wGR-LBD is identical to blue whales and killer whales and is 96.6–99.6% similar to the GR-LBD of polar bears, white whales and humans (Lühmann et al., 2020).

The highest concentrations of DEHP (25 and 50 μM) increased wTHRB-mediated luciferase activity by 22–27%, while the same concentrations decreased the activity by 23–33% in the presence of T3 (Fig. 3a). This indicates that DEHP acts as a partial agonist toward wTHRB with competitive antagonistic effects in the presence of a full agonist. A partial agonist/antagonist induces an antagonistic conformational change of the receptor, but it does not preclude an unstable agonist conformation (Bourguet et al., 2000). Similarly, DEHP showed both agonistic and antagonistic effects on the TH-dependent rat

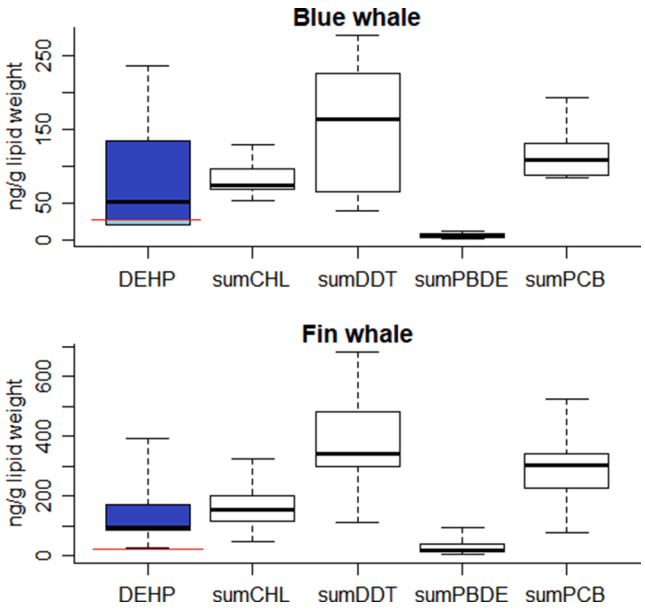

Fig. 2. Boxplot showing concentrations (ng/g lipid weight) of DEHP (blue) in comparison to the most abundant persistent organic pollutants (Tartu et al., 2020; Routti et al., 2019) in blubber from blue whales and fin whales sampled from Svalbard, 2014–2017. Since DEHP data was partly below the limit of detection, robust Regression on Order Statistics were used to estimate values (light blue) below the maximum limit of detection line marked in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

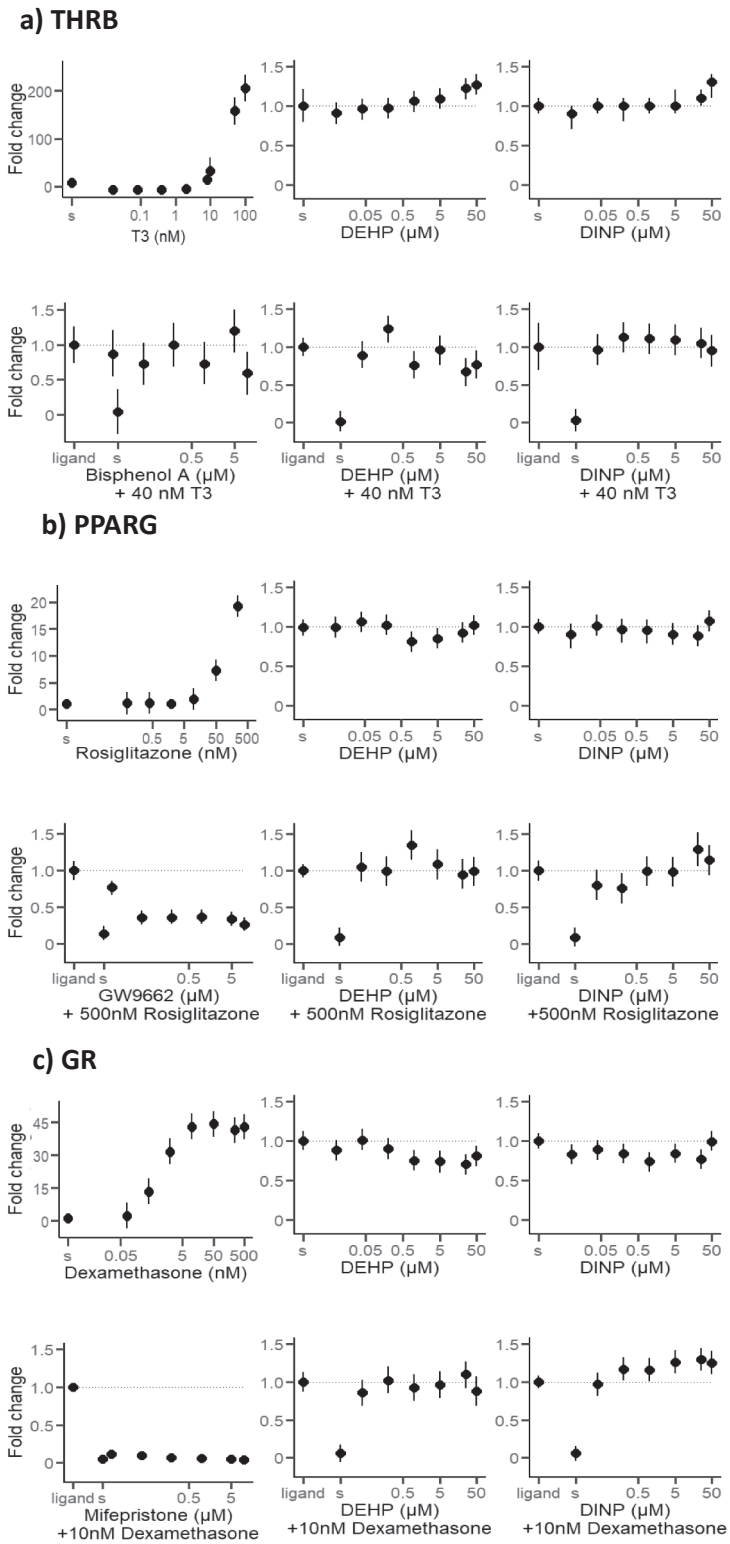

Fig. 3. Agonistic and antagonistic effects of known ligands, DEHP and DINP on fin whale THRB (a), PPARG (b) and GR (c) *in vitro*. Fold changes for agonistic effects are presented over solvent (s) and for agonistic effects over a known agonist. Data are derived from linear mixed-effect models, shown with 95% confidence intervals.

pituitary GH3 cell proliferation assay, suggesting that DEHP competes directly with T3 through TRs (Ghisari and Bonefeld-Jorgensen, 2009). DEHP also reduced luciferase activity mediated by THRB in transiently transfected African green monkey (*Chlorocebus aethiops*) kidney cells (Vero and CV-1) in the presence of T3, whereas agonistic effects were not observed (Shen et al., 2009; Sun et al., 2012).

DiNP increased wTHRB mediated luciferase activity by 30% (50 μM), which indicates that DiNP is a weak agonist of wTHRB (Fig. 3a). Antagonistic effects were not detected. In contrast to this study, DiNP inhibited TH-dependent GH3 rat pituitary cell proliferation stimulated by T3, and it also reduced the basal GH3 growth in the absence of T3 (Ghisari and Bonefeld-Jorgensen, 2009).

No significant agonistic or antagonistic effects by DEHP or DiNP were detected for wPPARG (Fig. 3b). Similarly, it has been found that DEHP did not show agonistic effects on human PPARG, which is identical to wPPARG (Lühmann et al., 2020), in transiently transfected COS1 and COS7 cells (Maloney and Waxman, 1999; Watt et al., 2016). Furthermore, molecular docking and *in vitro* assays indicate that human PPARG do not bind DEHP (Kratochvil et al., 2019). DEHP and DiNP did not modulate human PPARG activity in a dose-response manner in stably transfected U-2 OS human osteoblast cells either (Pereira-Fernandes et al., 2013). Interestingly, DiNP did increase human PPARG-mediated luciferase activity in transiently transfected HepG2 human liver carcinoma cells and molecular docking analyses showed the capability of DiNP to bind human PPARG at submicromolar concentrations (Pomatto et al., 2018).

DEHP and DiNP decreased basal luciferase activity mediated by wGR at several test concentrations, whereas no decrease in activity was observed in the presence of the agonist dexamethasone (Fig. 3c). In contrast, DEHP was a weak agonist and not an antagonist of GR in stably expressed MDA-kb2 human breast cells (Klopčič et al., 2015; Kolšek et al., 2014). *In silico* molecular docking studies indicate that human GR binds DEHP but not DiNP (Sarath Josh et al., 2016). The discrepancy in the results between whale and human GR is likely related to substitutions in their ligand binding domain that are 96% identical (Lühmann et al., 2020).

The tested compounds ($\leq$50 μM) did not show cytotoxicity, i.e. reduced membrane integrity or metabolic activity, in COS7 cells (Figure S1).

3.3. Toxicological implications

When converted to molar concentrations, DEHP was present in blubber biopsies at up to 1 μM concentration (one bowhead whale sample), whereas antagonistic and agonistic effects on wTHRB occurred consistently at 25 and 50 μM concentrations. This suggests that current DEHP concentrations do not modulate the function of the studied nuclear receptors in adipose tissue of blue whales, fin whales, bowhead whales or polar bears sampled from the Norwegian Arctic. DEHP exposure is more likely to modulate the function of THRB in the blubber of whales from southern latitudes such as the Mediterranean Sea, where concentrations up to 60 μM were reported (Baini et al., 2017). The results concerning the modulation of nuclear receptors by DEHP and DiNP are also relevant for human risk assessment, because the LBDs of wPPARG and wTHRB are identical to humans (Lühmann et al., 2020).

It should be noted that the presence of lipophilic phthalates in fat tissue do not give a complete picture of phthalate exposure in Arctic marine mammals. Phthalates go through phase I and phase II metabolism which render them into a more hydrophilic form that can be readily excreted. Metabolites of DnBP and DiBP were detected in few polar bear plasma samples (Table 2), and they have also been detected in cetacean liver (Rian et al., 2020). This suggests that phthalate metabolites may be formed and retained at least to some extent in Arctic marine mammals. In humans, phthalates (diesters) are hydrolysed predominantly by hepatic esterases to respective monoesters, which may undergo further hydrolysis or oxidation and a subsequent glucuronidation catalysed by UDP-glucuronocyltransferases (Benjamin et al., 2017; Silva et al., 2003). The low number of genes encoding UDP-glucuronocyltransferases in whales in comparison to humans (Kim et al., 2016b) may affect the ability of whales to conjugate phthalates with glucuronide.

4. Conclusions

This study shows that DEHP is present in marine mammals in the Norwegian Arctic; in blue whales and fin whales at concentrations similar to major legacy POPs. Phthalates are, however, likely to be at least partly biotransformed in cetaceans. The low detection rate of phthalates and their metabolites in polar bears suggests that phthalates are present or accumulate to a lesser extent in the polar bear food web, or that polar bears excrete them rapidly. Further analyses on phthalate metabolites in whales are warranted to get a more complete picture of phthalate exposure in Arctic marine mammals. This study also showed potential for DEHP and DiNP to modulate the transcriptional activity of wTHRB. Future studies should investigate whether phthalate exposure affects cellular processes in marine mammals.

CRediT authorship contribution statement

Heli Routti: Conceptualization, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Mikael Harju:** Methodology, Validation, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Katharina Lühmann:** Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Jon Aars:** Resources, Data curation, Writing - review & editing, Funding acquisition. **Amalie Ask:** Writing - original draft, Writing - review & editing. **Anders Goksøyr:** Resources, Writing - review & editing. **Kit M. Kovacs:** Resources, Writing - review & editing, Funding acquisition. **Christian Lydersen:** Resources, Data curation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106458.

References

Amelineau, F., Bonnet, D., Heitz, O., Mortreux, V., Harding, A.M.A., Karnovsky, N., Walkusz, W., Fort, J., Gremillet, D., 2016. Microplastic pollution in the Greenland Sea: background levels and selective contamination of planktivorous diving seabirds. Environ. Pollut. 219, 1131–1139. https://doi.org/10.1016/j.envpol.2016.09.017.

Amélineau, F., Bonnet, D., Heitz, O., Mortreux, V., Harding, A.M.A., Karnovsky, N., Walkusz, W., Fort, J., Grémillet, D., 2016. Microplastic pollution in the Greenland Sea: background levels and selective contamination of planktivorous diving seabirds. Environ. Pollut. 219, 1131–1139. https://doi.org/10.1016/j.envpol.2016.09.017.

Baak, J.E., Provencher, J.F., Mallory, M.L., 2020. Plastic ingestion by four seabird species in the Canadian Arctic: comparisons across species and time. Mar. Pollut. Bull. 158, 111386 https://doi.org/10.1016/j.marpolbul.2020.111386.

Baini, M., Martellini, T., Cincinelli, A., Campani, T., Minutoli, R., Panti, C., Finoia, M.G., Fossi, M.C., 2017. First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber. Anal. Meth. 9, 1512–1520. https://doi.org/10.1039/c6ay02674e.

Baken, K.A., Lambrechts, N., Remy, S., Mustieles, V., Rodríguez-Carrillo, A., Neophytou, C.M., Olea, N., Schoeters, G., 2019. A strategy to validate a selection of human effect biomarkers using adverse outcome pathways: proof of concept for phthalates and reproductive effects. Environ. Res. 175, 235–256. https://doi.org/10.1016/j.envres.2019.05.013.

Bakir, A., O'Connor, I.A., Rowland, S.J., Hendriks, A.J., Thompson, R.C., 2016. Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. Environ. Pollut. 219, 56–65. https://doi.org/10.1016/j.envpol.2016.09.046.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67.

Benjamin, S., Masai, E., Kamimura, N., Takahashi, K., Anderson, R.C., Faisal, P.A., 2017. Phthalates impact human health: epidemiological evidences and plausible mechanism of action. J. Hazard Mater. 340, 360–383. https://doi.org/10.1016/j.jhazmat.2017.06.036.

Bergmann, M., Lutz, B., Tekman, M.B., Gutow, L., 2017a. Citizen scientists reveal: Marine litter pollutes Arctic beaches and affects wild life. Mar. Pollut. Bull. 125, 535–540. https://doi.org/10.1016/j.marpolbul.2017.09.055.

Bergmann, M., Mützel, S., Primpke, S., Tekman, M.B., Trachsel, J., Gerdts, G., 2019. White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. Sci. Adv. 5, eaax1157. https://doi.org/10.1126/sciadv.aax1157.

Bergmann, M., Wirzberger, V., Krumpen, T., Lorenz, C., Primpke, S., Tekman, M.B., Gerdts, G., 2017b. High quantities of microplastic in arctic deep-sea sediments from the HAUSGARTEN observatory. Environ. Sci. Technol. 51, 11000–11010. https://doi.org/10.1021/acs.est.7b03331.

Berube, M., Palsboll, P., 1996. Identification of sex in cetaceans by multiplexing with three ZFX and ZFY specific primers. Mol Ecol 5, 283–287. https://doi.org/10.1111/j.1365-294x.1996.tb00315.x.

Bohlin-Nizzetto, P., Aas, W., Nikiforov, V, 2019.. Monitoring of environmental contaminants in air and precipitation. Annual report 2018. NILU report 11/2019. Norwegian Environment Agency M-1419|2019.

Bourguet, W., Germain, P., Gronemeyer, H., 2000. Nuclear receptor ligand-binding domains three-dimensional structures, molecular interactions and pharmacological implications. Trends Pharmacol Sci. 21, 381–388. https://doi.org/10.1016/S0165-6147(00)01548-0.

Cadepond, P.F., Ulmann, M., Baulieu, M., 1997. RU486 (mifepristone): mechanisms of action and clinical uses. Ann. Rev. Med. 48, 129–156.

Christensen-Dalsgaard, S.N., Aars, J., Andersen, M., Lockyer, C., Yoccoz, N.G., 2010. Accuracy and precision in estimation of age of Norwegian Arctic polar bears (*Ursus maritimus*) using dental cementum layers from known-age individuals. Polar Biol. 33, 589–597. https://doi.org/10.1007/s00300-009-0734-y.

Cousineau, D., Chartier, S., 2010. Outliers detection and treatment: a review. Int. J. Psychol. Res. 3, 58–67.

Cousins, I., Mackay, D., 2000. Correlating the physical–chemical properties of phthalate esters using the 'three solubility' approach. Chemosphere 41, 1389–1399. https://doi.org/10.1016/S0045-6535(00)00005-9.

Croll, D.A., Acevedo-Gutiérrez, A., Tershy, B.R., 2001. J.The diving behavior of blue and fin whales: is dive duration shorter than expected based on oxygen stores? Comp. Biochem. Physiol. A 129, 797–809. https://doi.org/10.1016/S1095-6433(01)00348-8.

Derocher, A.E., Wiig, O., Andersen, M., 2002. Diet composition of polar bears in Svalbard and the western Barents Sea. Polar Biol. 25, 448–452. https://doi.org/10.1007/s00300-002-0364-0.

ECPI. Focus on plasticisers, 2019, https://www.plasticisers.org/wp-content/uploads/2019/01/PLASTICISERS_LEAFLET_UPDATE_15012019_for_REPRINT_2019.pdf.

Eichler, C.M.A., Cohen Hubal, E.A., Little, J.C., 2019. Assessing human exposure to chemicals in materials, products and articles: the international risk management landscape for phthalates. Environ. Sci. Technol. 53, 13583–13597. https://doi.org/10.1021/acs.est.9b03794.

ExxonMobil Petroleum and Chemical B.V.B.A., 2014. Submission of Information on DINP CAS#68515-48-0, EC#271-090-9 as an Alternative to DEHP – Applications for Authorisation. https://echa.europa.eu/documents/10162/18074545/a4a_comment_46_1_attachment_en.pdf.

Fossi, M.C., Coppola, D., Baini, M., Giannetti, M., Guerranti, C., Marsili, L., Panti, C., de Sabata, E., Clo, S., 2014. Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: the case studies of the Mediterranean basking shark (Cetorhinus maximus) and fin whale (*Balaenoptera physalus*). Mar. Environ. Res. 100, 17–24. https://doi.org/10.1016/j.marenvres.2014.02.002.

Fossi, M.C., Marsili, L., Baini, M., Giannetti, M., Coppola, D., Guerranti, C., Caliani, I., Minutoli, R., Lauriano, G., Finoia, M.G., Rubegni, F., Panigada, S., Berube, M., Ramirez, J.U., Panti, C., 2016. Fin whales and microplastics: The Mediterranean Sea and the Sea of Cortez scenarios. Environ. Pollut. 209, 68–78. https://doi.org/10.1016/j.envpol.2015.11.022.

Gao, D.W., Wen, Z.D., 2016. Phthalate esters in the environment: a critical review of their occurrence, biodegradation, and removal during wastewater treatment processes. Sci. Total. Environ. 541, 986–1001. https://doi.org/10.1016/j.scitotenv.2015.09.148.

Gavrilchuk, K., Lesage, V., Ramp, C., Sears, R., Bérubé, M., Bearhop, S., Beauplet, G., 2014. Trophic niche partitioning among sympatric baleen whale species following the collapse of groundfish stocks in the Northwest Atlantic. Mar. Ecol. Prog. Ser. 497, 285–301. http://www.int-res.com/abstracts/meps/v497/p285-301/.

Ghisari, M., Bonefeld-Jorgensen, E.C., 2009. Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. Toxicol. Lett. 189, 67–77. https://doi.org/10.1016/j.toxlet.2009.05.004.

Gross, J., Pitt-Rivers, R., 1953. 3: 5: 3′-triiodothyronine 1 Isolation from thyroid gland and synthesis. Biochem. J. 53, 645.

Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling. J. Hazard Mater. 344, 179–199. https://doi.org/10.1016/j.jhazmat.2017.10.014.

Halsband, C., Herzke, D., 2019. Plastic litter in the European Arctic: what do we know? Emerg. Contam. 5, 308–318. https://doi.org/10.1016/j.emcon.2019.11.001.

Hansen, E., Nilsson, N.H., Lithner, D., Lassen, C., 2013. Hazardous substances in plastic materials. TA-3017 https://www.miljodirektoratet.no/globalassets/publikasjoner/klif2/publikasjoner/3017/ta3017.pdf.

Hart, L.B., Beckingham, B., Wells, R.S., Alten Flagg, M., Wischusen, K., Moors, A., Kucklick, J., Pisarski, E., Wirth, E., 2018. Urinary phthalate metabolites in common bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, FL, USA. GeoHealth 2, 313–326. https://doi.org/10.1029/2018gh000146.

Helsel, D.R., 2011. Statistics for Censored Environmental Data Using Minitab and R. John Wiley & Sons.

Holland, M., 2018. Socio-economic assessment of phthalates. OECD Environment Working Papers No. 133.

Hu, X., Gu, Y., Huang, W., Yin, D., 2016. Phthalate monoesters as markers of phthalate contamination in wild marine organisms. Environ. Pollut. 218, 410–418. https://doi.org/10.1016/j.envpol.2016.07.020.

Ikonomou, M.G., Kelly, B.C., Blair, J.D., Gobas, F.A.P.C., 2012. An interlaboratory comparison study for the determination of dialkyl phthalate esters in environmental and biological samples. Environ. Toxicol. Chem. 31, 1948–1956. https://doi.org/10.1002/etc.1912.

Jeong, J.Y., Lee, J.H., Kim, E.Y., Kim, P.G., Kho, Y.L., 2011. Determination of phthalate metabolites in human serum and urine as biomarkers for phthalate exposure using column-switching LC-MS/MS. Saf. Health Work 2, 57–64. https://doi.org/10.5491/SHAW.2011.2.1.57.

Kanhai, D.K., Gardfeldt, K., Krumpen, T., Thompson, R.C., O'Connor, I., 2020. Microplastics in sea ice and seawater beneath ice floes from the Arctic Ocean. Sci. Rep. 10, 5004. https://doi.org/10.1038/s41598-020-61948-6.

Katsikantami, I., Sifakis, S., Tzatzarakis, M.N., Vakonaki, E., Kalantzi, O.I., Tsatsakis, A. M., Rizos, A.K., 2016. A global assessment of phthalates burden and related links to health effects. Environ. Int. 97, 212–236. https://doi.org/10.1016/j.envint.2016.09.013.

Kelly, B.C., Ikonomou, M.G., Blair, J.D., Morin, A.E., Gobas, F.A.P.C., 2007. Food web specific biomagnification of persistent organic pollutants. Science 317, 236–239. https://doi.org/10.1126/science.1138275.

Kim, J., Gobas, F.A.P.C., Arnot, J.A., Powell, D.E., Seston, R.M., Woodburn, K.B., 2016a. Evaluating the roles of biotransformation, spatial concentration differences, organism home range, and field sampling design on trophic magnification factors. Sci. Total. Environ. 551–552, 438–451. https://doi.org/10.1016/j.scitotenv.2016.02.013.

Kim, S., Cho, Y.S., Kim, H.-M., Chung, O., Kim, H., Jho, S., Seomun, H., Kim, J., Bang, W. Y., Kim, C., An, J., Bae, C.H., Bhak, Y., Jeon, S., Yoon, H., Kim, Y., Jun, J., Lee, H., Cho, S., Uphyrkina, O., Kostyria, A., Goodrich, J., Miquelle, D., Roelke, M., Lewis, J., Yurchenko, A., Bankevich, A., Cho, J., Lee, S., Edwards, J.S., Weber, J.A., Cook, J., Kim, S., Lee, H., Manica, A., Lee, I., O'Brien, S.J., Bhak, J., Yeo, J.-H., 2016b. Comparison of carnivore, omnivore, and herbivore mammalian genomes with a new leopard assembly. Genome Biol. 17, 211. https://doi.org/10.1186/s13059-016-1071-4.

Klopčič, I., Kolšek, K., Dolenc, M.S., 2015. Glucocorticoid-like activity of propylparaben, butylparaben, diethylhexyl phthalate and tetramethrin mixtures studied in the MDA-kb2 cell line. Toxicol. Lett. 232, 376–383. https://doi.org/10.1016/j.toxlet.2014.11.019.

Kolšek, K., Gobec, M., Mlinarič Raščan, I., Sollner Dolenc, M., 2014. Molecular docking revealed potential disruptors of glucocorticoid receptor-dependent reporter gene expression. Toxicol. Lett. 226, 132–139. https://doi.org/10.1016/j.toxlet.2014.01.047.

Kratochvil, I., Hofmann, T., Rother, S., Schlichting, R., Moretti, R., Scharnweber, D., Hintze, V., Escher, B.I., Meiler, J., Kalkhof, S., von Bergen, M., 2019. Mono(2-ethylhexyl) phthalate (MEHP) and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP) but not di(2-ethylhexyl) phthalate (DEHP) bind productively to the peroxisome proliferator-activated receptor gamma. Rap. Comm. Mass Spectr. 33, 75–85. https://doi.org/10.1002/rcm.8258.

Kvadsheim, P.H., Folkow, L.P., Blix, A.S., 1996. Thermal conductivity of minke whale blubber. J. Therm. Biol. 21, 123–128. https://doi.org/10.1016/0306-4565(95)00034-8.

Lee, L. NADA: Nondetects and data analysis for environmental data. R package version 16-1 2017:15-16.

Leesnitzer, L.M., Parks, D.J., Bledsoe, R.K., Cobb, J.E., Collins, J.L., Consler, T.G., Davis, R.G., Hull-Ryde, E.A., Lenhard, J.M., Patel, L., 2002. Functional consequences of cysteine modification in the ligand binding sites of peroxisome proliferator activated receptors by GW9662. Biochemistry 41, 6640–6650.

Lefterova, M.I., Haakonsson, A.K., Lazar, M.A., Mandrup, S., 2014. PPARγ and the global map of adipogenesis and beyond. Trends Endocrin. Metab. 25, 293–302. https://doi.org/10.1016/j.tem.2014.04.001.

Lehmann, J.M., Moore, L.B., Smith-Oliver, T.A., Wilkison, W.O., Willson, T.M., Kliewer, S.A., 1995. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). J. Biol. Chem. 270, 12953–12956.

Lille-Langøy, R., Goldstone, J.V., Rusten, M., Milnes, M.R., Male, R., Stegeman, J.J., Blumberg, B., Goksøyr, A., 2015. Environmental contaminants activate human and polar bear (*Ursus maritimus*) pregnane X receptors (PXR, NR1I2) differently. Toxicol. Appl. Pharmacol. 284, 54–64. https://doi.org/10.1016/j.taap.2015.02.001.

Lühmann, K., Lille-Langøy, R., Øygarden, L., Kovacs, K.M., Lydersen, C., Goksøyr, A., Routti, H., 2020. Environmental pollutants modulate transcriptional activity of nuclear receptors of whales in vitro. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.9b06952.

Lusher, A.L., Tirelli, V., O'Connor, I., Officer, R., 2015. Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. Sci. Rep. 5 https://doi.org/10.1038/srep14947.

Lydersen, C., Vacquié-Garcia, J., Heide-Jørgensen, M.P., Øien, N., Guinet, C., Kovacs, K. M., 2020. Autumn movements of fin whales (*Balaenoptera physalus*) from Svalbard, Norway, revealed by satellite tracking. Sci. Rep. 10, 16966. https://doi.org/10.1038/s41598-020-73996-z.

Mackintosh, C.E., Maldonado, J., Jing, H.W., Hoover, N., Chong, A., Ikonomou, M.G., Gobas, F., 2004. Distribution of phthalate esters in a marine aquatic food web: comparison to polychlorinated biphenyls. Environ. Sci. Technol. 38, 2011–2020. https://doi.org/10.1021/es034745r.

Maindonald, J., Braun, W., 2015. DAAG: data analysis and graphics data and functions [Software]. URL http://CRANR-project.org/package=DAAG (R package version 122).

Malcolm Richard, G., Mario, M., Javier, T., Susana, T., 2011. Optimization of the recovery of plastics for recycling by density media separation cyclones. Resour. Conserv. Recyc. 55, 472–482. https://doi.org/10.1016/j.resconrec.2010.12.010.

Maloney, E.K., Waxman, D.J., 1999. Trans-activation of PPAR[alpha] and PPAR[gamma] by structurally diverse environmental chemicals. Toxicol. Appl. Pharmacol. 161, 209–218. http://www.sciencedirect.com/science/article/B6WXH-45FK0CV-X/2/2d07d68431a8e69068f3f650204446d1.

Millard, S.P., Kowarik, M.A., Imports, M., 2018. Package 'EnvStats'. Package for environmental statistics version 2018;2.

Moore, R.C., Loseto, L., Noel, M., Etemadifar, A., Brewster, J.D., MacPhee, S., Bendell, L., Ross, P.S., 2020. Microplastics in beluga whales (*Delphinapterus leucas*) from the Eastern Beaufort Sea. Mar. Pollut. Bull. 150, 110723 https://doi.org/10.1016/j.marpolbul.2019.110723.

Morgana, S., Ghigliotti, L., Estévez-Calvar, N., Stifanese, R., Wieckzorek, A., Doyle, T., Christiansen, J.S., Faimali, M., Garaventa, F., 2018. Microplastics in the Arctic: a case study with sub-surface water and fish samples off Northeast Greenland. Environ. Pollut. 242, 1078–1086. https://doi.org/10.1016/j.envpol.2018.08.001.

Moriyama, K., Tagami, T., Akamizu, T., Usui, T., Saijo, M., Kanamoto, N., Hataya, Y., Shimatsu, A., Kuzuya, H., Nakao, K., 2002. Thyroid hormone action is disrupted by bisphenol A as an antagonist. J. Clin. Endocrin. Metab. 87, 5185–5190.

Mullur, R., Liu, Y.-Y., Brent, G.A., 2014. Thyroid hormone regulation of metabolism. Physiol. Rev. 94, 355–382. https://doi.org/10.1152/physrev.00030.2013.

Nagorka, R., Koschorreck, J., 2020. Trends for plasticizers in German freshwater environments - evidence for the substitution of DEHP with emerging phthalate and non-phthalate alternatives. Environ. Pollut. 262, 114237 https://doi.org/10.1016/j.envpol.2020.114237.

Net, S., Sempere, R., Delmont, A., Paluselli, A., Ouddane, B., 2015. Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. Environ. Sci. Technol. 49, 4019–4035. https://doi.org/10.1021/es505233b.

Nielsen, N., Laidre, K., Stanbak Larsen, R., Heide-Jørgensen, M.P., 2015. Identification of potential foraging areas for bowhead whales in Baffin Bay and adjacent waters. Arctic 68. https://doi.org/10.14430/arctic4488.

Paluselli, A., Fauvelle, V., Galgani, F., Sempéré, R., 2019. Phthalate release from plastic fragments and degradation in seawater. Environ. Sci. Technol. 53, 166–175. https://doi.org/10.1021/acs.est.8b05083.

Pereira-Fernandes, A., Demaegdt, H., Vandermeiren, K., Hectors, T.L., Jorens, P.G., Blust, R., Vanparys, C., 2013. Evaluation of a screening system for obesogenic compounds: screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect. Plos One 8, e77481. https://doi.org/10.1371/journal.pone.0077481.

Pérez-Jorge, S., Tobeña, M., Prieto, R., Vandeperre, F., Calmettes, B., Lehodey, P., Silva, M.A., 2020. Environmental drivers of large-scale movements of baleen whales in the mid-North Atlantic Ocean. Divers. Distrib. 26, 683–698. https://doi.org/10.1111/ddi.13038.

Pomatto, V., Cottone, E., Cocci, P., Mozzicafreddo, M., Mosconi, G., Nelson, E.R., Palermo, F.A., Bovolin, P., 2018. Plasticizers used in food-contact materials affect adipogenesis in 3T3-L1 cells. J. Steroid Biochem. 178, 322–332. https://doi.org/10.1016/j.jsbmb.2018.01.014.

Provencher, J.F., Avery-Gomm, S., Braune, B.M., Letcher, R.J., Dey, C.J., Mallory, M.L., 2020. Are phthalate ester contaminants in northern fulmar preen oil higher in birds that have ingested more plastic? Mar. Pollut. Bull. 150.

Remberger, M., Kaj, L., Hansson, K., Andersson, H., Brorström-Lundén, E., Lunder, H., Schlabach, M., 2013. Selected plasticisers and additional sweeteners in the Nordic environment. TemaNord. Nordic Council of Ministers 2013:505.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reyes, J.M., Price, P.S., 2018. Temporal trends in exposures to six phthalates from biomonitoring data: implications for cumulative risk. Environ. Sci. Technol. 52, 12475–12483. https://doi.org/10.1021/acs.est.8b03338.

Rian, M.B., Vike-Jonas, K., Gonzalez, S.V., Ciesielski, T.M., Venkatraman, V., Lindstrøm, U., Jenssen, B.M., Asimakopoulos, A.G., 2020. Phthalate metabolites in harbor porpoises (*Phocoena phocoena*) from Norwegian coastal waters. Environ. Int. 137, 105525 https://doi.org/10.1016/j.envint.2020.105525.

Routti, H., Lille-Langøy, R., Berg, M.K., Fink, T., Harju, M., Kristiansen, K., Rostkowski, P., Rusten, M., Sylte, I., Øygarden, L., Goksøyr, A., 2016. Environmental chemicals modulate polar bear (*Ursus maritimus*) peroxisome proliferator-activated receptor gamma (PPARG) and adipogenesis in vitro. Environ. Sci. Technol. 50, 10708–10720. https://doi.org/10.1021/acs.est.6b03020.

Routti, H., Lydersen, C., Kovacs Kit, M., Tartu, S., Götsch, A., Fisk, A., 2019. Pollutants and stable isotopes in Northeast Atlantic blue whales and fin whales [Data set]. Norwegian Polar Institute. https://doi.org/10.21334/npolar.2019.9f0a9b32.

Routti, H., Harju, M., Lühmann, K., Aars, J., Goksøyr, A., Kovacs, K.M., Lydersen, C., 2021. Concentrations of phthtalates and their potential to transactivate nuclear receptors in marine mammals from the Norwegian Arctic [Data set]. Norwegian Polar Institute. doi.org/10.21334/npolar.2021.5499f1ad.

Sarath Josh, M.K., Pradeep, S., Vijayalekshmy Amma, K.S., Sudha Devi, R., Balachandran, S., Sreejith, M.N., Sailas, B., 2016. Human ketosteroid receptors interact with hazardous phthalate plasticizers and their metabolites: an in silico study. J. Appl. Toxicol. 36, 836–843. https://doi.org/10.1002/jat.3221.

Schreer, A., Tinson, C., Sherry, J.P., Schirmer, K., 2005. Application of Alamar blue/5-carboxyfluorescein diacetate acetoxymethyl ester as a noninvasive cell viability assay in primary hepatocytes from rainbow trout. Anal. Biochem. 344, 76–85.

Shen, O., Du, G., Sun, H., Wu, W., Jiang, Y., Song, L., Wang, X., 2009. Comparison of in vitro hormone activities of selected phthalates using reporter gene assays. Toxicol. Lett. 191, 9–14.

Silva, M.J., Malek, N.A., Hodge, C.C., Reidy, J.A., Kato, K., Barr, D.B., Needham, L.L., Brock, J.W., 2003. Improved quantitative detection of 11 urinary phthalate metabolites in humans using liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry. J. Cromatogr. B 789, 393–404. https://doi.org/10.1016/S1570-0232(03)00164-8.

Sun, H., Si, C., Bian, Q., Chen, X., Chen, L., Wang, X., 2012. Developing in vitro reporter gene assays to assess the hormone receptor activities of chemicals frequently detected in drinking water. J Appl. Toxicol. 32, 635–641. https://doi.org/10.1002/jat.1790.

Tartu, S., Fisk, A.T., Götsch, A., Kovacs, K.M., Lydersen, C., Routti, H., 2020. First assessment of pollutant exposure in two balaenopterid whale populations sampled in the Svalbard Archipelago, Norway. Sci. Total. Environ. 718, 137327 https://doi.org/10.1016/j.scitotenv.2020.137327.

Tsurufuji, S., Sugio, K., Takemasa, F., 1979. The role of glucocorticoid receptor and gene expression in the anti-inflammatory action of dexamethasone. Nature 280, 408.

Vegiopoulos, A., Herzig, S., 2007. Glucocorticoids, metabolism and metabolic diseases. Mol. Cell. Endocrinol. 275, 43–61. https://doi.org/10.1016/j.mce.2007.05.015.

Ventrice, P., Ventrice, D., Russo, E., De Sarroa, G., 2013. Phthalates: European regulation, chemistry, pharmacokinetic and related toxicity. Environ. Toxicol. Phar. 36, 88–96. https://doi.org/10.1016/j.etap.2013.03.014.

Vorkamp, K., Dam, M., Riget, F., Fauser, P., Bossi, R., Hansen, A.B., 2004. Screening of "new" contaminants in the marine environment of Greenland and the Faroe Islands. National Environmental Research Institute. NERI Technical Report. Denmark. http://technical-reports.dmu.dk.

Watt, J., Webster, T.F., Schlezinger, J.J., 2016. Generalized concentration addition modeling predicts mixture effects of environmental PPARγ agonists. Toxicol. Sci. 153, 18–27. https://doi.org/10.1093/toxsci/kfw100.

Wilkes, C.E., Summers, J.W., Daniels, C.A., Berard, M.T., 2005. PVC Handbook. Hanser, Munich; Cincinnati.

Xie, Z., Ebinghaus, R., Temme, C., Lohmann, R., Caba, A., Ruck, W., 2007. Occurrence and air-sea exchange of phthalates in the Arctic. Environ. Sci. Technol. 41, 4555–4560.

Zhang, J., Zhang, J., Liu, R., Gan, J., Liu, J., Liu, W., 2015. Endocrine-disrupting effects of pesticides through interference with human glucocorticoid receptor. Environ. Sci. Technol. 50, 435–443.

Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Meth. Ecol. Evolut. 1, 3–14.