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Physiologically-based pharmacokinetic modelling of immune, reproductive and carcinogenic effects from contaminant exposure in polar bears (*Ursus maritimus*) across the Arctic

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ABSTRACT

Polar bears (*Ursus maritimus*) consume large quantities of seal blubber and other high trophic marine mammals and consequently have some of the highest tissue concentrations of organohalogen contaminants (OHCs) among Arctic biota. In the present paper we carried out a risk quotient (RQ) evaluation on OHC-exposed polar bears harvested from 1999 to 2008 and from 11 circumpolar subpopulations spanning from Alaska to Svalbard in order to evaluate the risk of OHC-mediated reproductive effects (embryotoxicity, teratogenicity), immunotoxicity and carcinogenicity (genotoxicity). This RQ evaluation was based on the Critical Body Residue (CBR) concept and a Physiologically-Based Pharmacokinetic Modelling (PBPK) approach using OHC concentrations measured in polar bear adipose or liver tissue. The range of OHC concentrations within polar bear populations were as follows for adipose, sum polychlorinated biphenyls ∑PCBs (1797–10,537 ng/g lw), sum methylsulphone-PCB ∑$MeSO_2$-PCBs (110–672 ng/g lw), sum chlordanes ∑CHLs (765–3477 ng/g lw), α-hexachlorocyclohexane α-HCH (8.5–91.3 ng/g lw), β-hexachlorocyclohexane β-HCH (65.5–542 ng/g lw), sum chlorbenzenes ∑ClBzs (145–304 ng/g lw), dichlorodiphenyltrichloroethane ∑DDTs (31.5–206 ng/g lw), dieldrin (69–249 ng/g lw), polybrominated diphenyl ethers ∑PBDEs (4.6–78.4 ng/g lw). For liver, the perfluorooctanesulfonic acid (PFOS) concentrations ranged from 231-2792 ng/g ww. The total additive RQ from all OHCs ranged from 4.3 in Alaska to 28.6 in East Greenland bears for effects on reproduction, immune health and carcinogenicity, highlighting the important result that the toxic effect threshold (i.e. RQ > 1) was exceeded for all polar bear populations assessed. PCBs were the main contributors for all three effect categories, contributing from 70.6% to 94.3% of the total risk and a RQ between 3.8–22.5. ∑$MeSO_2$–PCBs were the second highest effect contributor for reproductive and immunological effects ($0.17 < RQ < 1.4$), whereas PFOS was the second highest effect contributor for carcinogenic (genotoxic) effects ($0.35 < RQ < 2.5$). The results from this study corroborate and lend further support to previous assessments of the possible adverse health effects of exposure to known and measured OHCs in polar bears. We therefore suggest that Critical Daily Doses (CDD) should be investigated in "*ex vivo*" dose–response studies on polar bears to replace laboratory studies on rats (*Rattus rattus*) to reveal whether high RQs are maintained.

1. Introduction

It is a challenge to assess the biological and potentially toxic effects in wildlife as a result of exposure to and mediation by organohalogen contaminants (OHCs). Such OHCs include the legacy persistent organic pollutants (POPs), as well as the more recently emerged POPs including brominated flame retardants (BFRs, e.g. polybrominated diphenyl ethers (PBDEs)) and perfluorinated compounds (PFCs) and especially the highly bioaccumulative

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URL: http://pure.au.dk/portal/en/persons/rune-dietz(86cb72ef-d48d-4395-94ee-b1c0bc3e33b5)/more.html (R. Dietz).

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perfluorooctane sulphonate (PFOS). Often, the life history of an individual study animal is unknown and usually only a cross-sectional examination is obtained of concentrations of known contaminants in selected tissues or blood plasma. Furthermore, the disruptive influences of environmental exposure to a "cocktail" of persistent and bioaccumulating chemicals on physiological systems are difficult to interpret due to the unknown agonistic and antagonistic interactions of the various pollutants.

Studies on polar bears (*Ursus maritimus*) have shown that various OHCs may affect vitamin concentrations (Braathen et al., 2004 Skaare et al., 2001) as well as hormones (Braathen et al., 2004; Haave et al., 2003; Oskam et al., 2003, 2004; Skaare et al., 2001), all of which are important for growth and reproduction. High OHC concentrations have also been associated with a reduction in size of sexual organs in polar bears (Sonne et al., 2006a, 2007a) leading to potential disruption of their reproductive capacity (Sonne et al., 2009). Sonne et al. (2006a) likewise showed that baculum bone mineral densities decreased with increasing chlordanes, DDTs, and HCB in both subadults and adult male polar bears which was recently supported by a study showed a decline in baculum bone mineral density from central Canada towards East Greenland along the increasing gradient of PCBs covering eight polar bear management areas from eastern Canada to East Greenland (Sonne et al., 2015). Substantial reporting on OHC concentrations in polar bears from East Greenland and Svalbard has highlighted the association between OHC exposure and changes in various immune and endocrine related biomarker responses (Letcher et al., 2010). Although these studies are correlative and cannot establish a clear cause–effect relationship, a weight of evidence approach combined with evidence from controlled laboratory and captive feeding studies suggests these (potential health) effects are likely the result of exposure to environmental chemicals (e.g., Braathen et al., 2004; Fisk et al., 2005; Haave et al., 2003; Kirkegaard et al., 2005; Lie et al., 2004, 2005; Muir et al., 2006; Oskam et al., 2003, 2004; Sonne et al., 2004, 2005a,b, 2006a,b, 2007a,b, 2008a; Verreault et al., 2008).

In the two Arctic POP assessments of the Arctic Monitoring and Assessment Programme (AMAP 1998, 2004), toxic effects from OHC body residues in Arctic marine mammals were extrapolated from laboratory animals in order to evaluate sub-lethal chronic toxicity. Recently, Letcher et al. (2010) reviewed OHC exposure and effects in Arctic wildlife and fish showing that extrapolation of effects in laboratory experiments to wildlife has inherent weaknesses. Among these are differences in species specific sensitivity to OHCs, metabolism, life and reproductive cycle and the fact that laboratory animals are most often exposed to a single individual compounds and congeners (e.g. PCB congeners) at high doses for short periods of time. In contrast, wildlife species are typically exposed to low doses of a variety of OHCs over their lifetimes. Arctic species are known to be more sensitive to the reproductive effects of contaminants than laboratory animals (Sandell, 1990). On the other hand, an understanding of the linkages between contaminants and potential health effects is most likely to come from studies in laboratory animals. Once these linkages are established in laboratory animal trials they can inform our understanding of the linkages between contaminants and health effects in exposed wild animals.

The weight of evidence of correlative associations of OHC exposure levels and various physiological and biochemical endpoints (*e.g.* endocrine and immune) supports the case that certain “hot spot” populations of polar bears, including those from East Greenland and Svalbard, may be at greater health risk. However, comprehensive reviews of OHC exposure and effects in Arctic wildlife, including polar bears, concluded that there remains a virtual absence of data demonstrating direct OHC-mediated cause-effect (Letcher et al., 2010; Sonne, 2010). In addressing the concerns outlined by Letcher et al. (2010), the present study evaluates the possible linkages of exposure to known OHCs and effects on reproduction, the immune system and carcinogenicity (genotoxicity) in polar bears across the Arctic using the most recent OHC geographical trend data from 2006 to 2008 from McKinney et al. (2011) and PFOS data from Letcher (unpublished). To achieve this, we carried out Risk Quotient (RQ) calculations based on the ratio of OHC concentrations in polar bear tissues to estimate Critical Body Residues (CBRs). Critical body residues were calculated from Critical Daily Doses (CDD) determined in laboratory studies on rats, using a physiologically-based pharmacokinetic (PBPK) model approach (see Supplementary information for further information on this concept). The PBPK model we used was developed by Cahill et al. (2003) and has been used for various contaminant purposes in *e.g.* humans (Redding et al., 2008). This approach allowed an estimation of the risk of possible adverse effects in polar bears by comparing OHC tissue residues with determined CBR values obtained from the most recent reviews of primarily orally exposed laboratory rats (*Rattus rattus*) (EFSA, 2008; Nielsen et al., 2006; US EPA, 2008). Such modelling has previously been conducted for a single population in East Greenland over three time periods (Sonne et al., 2009).

Such a cross-species analysis of CBRs assumes comparable sensitivity and mode of action by which OHCs disrupt the endocrine systems between species. Indeed, such assumptions are routinely made in areas such as pharmacology, where drugs are initially tested on model organisms, or in toxicology where safe drinking water standards are set based on effects found in laboratory models. Hence, we believe that the present study is the closest we can presently get to evaluating the risk and provide a ranking of OHC severity to wild polar bears from measured OHC exposure, knowing that not all POPs have been included in the complex cocktail that polar bears are exposed to due to a lack of reliable knowledge on CBRs for these substances.

2. Materials and methods

2.1. Chemical analyses

Organohalogen concentrations for polar bear subcutaneous adipose and liver tissues were based on the two most recent trans-Arctic studies of legacy and recently emerged OHCs (Letcher, unpublished; McKinney et al., 2011). Polar bears (total $n=165$) were sampled from 2005 to 2008 in 11 subpopulations: Alaska–Chukchi/Bering Sea (AL), southern Beaufort Sea (SBS), northern Beaufort Sea (NBS), Gulf of Boothia (GB), Lancaster/Jones Sound (LJS), Baffin Bay (BB), Davis Strait (DS), western Hudson Bay (WHB), southern Hudson Bay (SHB), East Greenland (EG) and Svalbard (SV) (Table 1; Fig. 1). Details on the analytical methods of PFOS are provided in Supplementary material, whereas other analytical methods the remaining OHCs are given in McKinney et al. (2011).

2.2. Critical Body Residue (CBR) and effect data

We used the same approach for estimating CBR data as used in Sonne et al. (2009). Critical body residues were calculated based on CDD determined in studies on rats using a physiologically-based pharmacokinetic (PBPK) model developed by Cahill et al. (2003). However, some of the CDDs for reproduction were updated and additional data on CBRs for immune suppression and carcinogenetic (genotoxic) effects were also calculated (Table 2, Supplementary information Table S1). Information on CDDs was not always available from the exact same chemical congener mixture, so here the closest chemical substance/congener was used (Supplementary information Table S1). A description of the method

Table 1
Geometric means and 95% confidence intervals of brominated and chlorinated contaminants and metabolites in adipose (ng/g lipid weight) as well as PFOS in liver (ng/g ww) from 11 polar bears subpopulations collected between 2005–2008 used for modelling Risk Quotients in the present study. OHC data were obtained from McKinney et al. (2011) and PFOS data from and Letcher (unpublished). All concentrations were age and sex corrected to a 7 yr old female polar bear. Recent population trends IUCN Polar Bear Specialist Group are listed for the 11 management areas (Vongraven and York, 2014).

Population trends	**Alaska** ***D.d***	**S. Beaufort Sea** ***Declining***	**N. Beaufort Sea** ***Stable***	**Gulf of Boothia** ***Stable***	**Lancaster/ Jones Sound** ***D.d***	**Baffin Bay** ***Declining***	**Davis Strait** ***Stable***	**W. Hudson Bay** ***Declining***	**S. Hudson Bay** ***Stable***	**E. Greenland** ***D.d***	**Svalbard** ***D.d***
∑PCB	1797 1013–3187	3688 2600–5232	5541 4518–6797	2445 1599–3739	2598 2005–3366	3211 2305–4472	4674 2693–8114	4634 3072–6992	5523 4617–6608	10537 8751–12687	5137 2854–9246
PFOS	396 289–541	714 531–961	701 565–870	554 377–814	746 557–998	1187 898–1568	580 403–835	857 634–1158	1633 1231–2167	2792 2203–3537	231 174–307
∑CHL	765 529–1106	1268 926–1736	1982 1555–2525	1824 1135–2930	1130 788–1619	2167 1523–3083	2135 1383–3298	3477 2386–5068	2166 1604–2924	1732 1292–2321	1196 793–1802
β-HCH	367 267–504	249 190–326	307 250–379	542 361–815	238 174–324	137 101–186	202 139–294	141 102–196	113 87–147	75.1 58.1–96.8	65.5 45.8–93.6
∑MeSO2-PCB	110 80–153	332 176–306	264 213–327	144 94.7–219	184 134–253	230 169–314	218 149–320	206 147–286	238 183–310	672 520–870	222 155–319
∑ClBz	156 110–221	145 106–197	237 199–282	304 231–400	234 194–283	266 205–344	255 149–435	221 174–279	171 150–194	189 141–253	166 115–240
∑DDT	90 68.0–119	81.8 60.5–110	93.7 79.9–110	31.5 19.8–49.7	64 44.1–92.7	179 125–257	104 60.0–181	88.1 54.8–141	152 127–182	206 155–273	119 75.0–187
Dieldrin	59.8 34.4–104	69 47.5–100	126 104–153	150 111–202	115 90.8–146	197 161–241	183 103–323	244 185–322	143 111–185	156 121–200	143 107–190
α-HCH	17.9 12.8–24.8	38.9 28.4–53.1	63.5 56.7–71.1	91.3 70.3–119	46.7 34.3–63.4	32.7 27.8–38.3	34.9 23.4–51.9	48.9 38.1–62.8	65.7 53.7–80.3	11.7 9.7–14.0	8.5 6.8–10.6
∑PBDE	4.6 3.4–6.2	5.8 4.3–7.9	8.8 7.7–10.0	7 4.5–10.7	6.8 5.1–9.0	14 11.4–17.0	27.1 16.7–43.4	38.6 27.5–54.0	78.4 65.6–93.6	43.2 37.5–49.8	44.4 32.5–60.6

(D.d.: Data deficient)

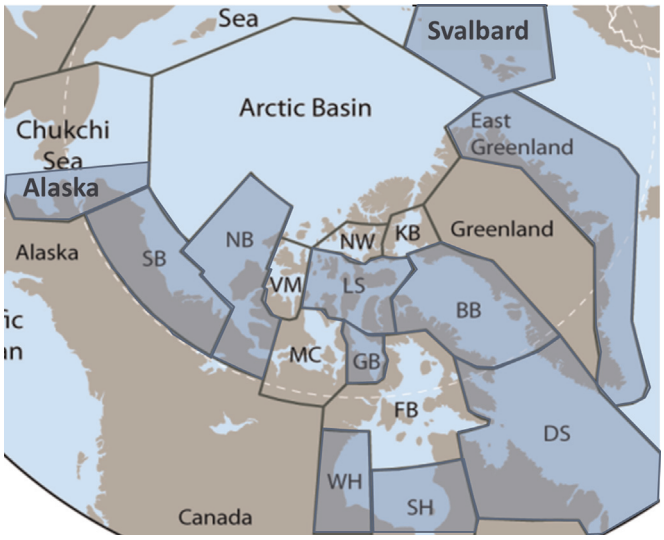

Fig. 1. Map of Arctic and Subarctic IUCN polar bear management areas (blue hatched areas) from which the Risk Quotients were calculated: Alaska (Chukchi Sea), SB; Southern Beaufort Sea, NB: Northern Beaufort Sea, GB: Golf of Boothia, LS: Lancaster Sound/Jones Sound, WH: Western Hudson Bay, BB: Baffin Bay/Northern Baffin Island, DS: Davis Strait/Southern Baffin Island, East Greenland, and Svalbard area. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Source: IUCN/Polar Bear Specialist Group.

used to calculate the CBR using the PBPK model is likewise provided in the Supplementary information.

3. Results and discussion

A detailed description on the geographical patterns of OHCs in the polar bears from 2005 to 2008 previously published by McKinney et al. (2011) was used in the present modelling (Table 1). For the PFOS and other PFCs, graphical patterns from an earlier dataset has been described in detail by Smithwick et al. (2005), but to secure comparability we extracted more recent PFOS data (Letcher, unpublished) from the same animals as those reported by McKinney et al. (2011). The range of adipose OHC geometric mean concentrations among polar bear populations were as follows: ∑PCBs (1797–10,537 ng/g lw), ∑$MeSO_2$–PCBs (110–672 ng/g lw), ∑CHLs (765–3477 ng/g lw), α-HCH (8.5–91.3 ng/g lw), β-HCH (65.5–542 ng/g lw), ∑ClBzs (145–304 ng/g lw), ∑DDTs (31.5–206 ng/g lw), dieldrin (69–249 ng/g lw), and ∑PBDEs (4.6–78.4 ng/g lw). For liver, PFOS concentrations ranged from 231 to 2792 ng/g ww. The geographical pattern of the contaminant loads presented in Table 1 is described in detail by McKinney et al. (2011) and in the Supplementary information. These geographical patterns are likewise reflected in the RQ calculated and presented below. RQs for polar bears were calculated from geometric mean body residues of bears adjusted to 7 yr old females as presented by McKinney et al. (2011). Hereafter, RQs were estimated for each OHC group (PCBs, DDTs, dieldrin, oxychlordane, HCHs, ClBz, PBDEs and PFOS) from which CBRs could be found in the literature (Table 1, Figs. 2–4, SI Tables 1–4).

3.1. Reproductive effects

The sum RQ for effects on the reproductive system of all investigated OHCs were > 1 for all polar bear populations and ranged from 4.3 in the Chukchi Sea/Alaska to 24.7 in East Greenland (Fig. 2). ∑PCBs were the main contributor to the total RQ within all regions (87–94%), alone showing a RQ > 1 for all bear populations, with the highest risk in East Greenland (∑PCBs RQ=22.5) and lowest in the Chukchi Sea/Alaska (∑PCBs RQ=3.8) (Fig. 2, Supplementary information Table S2). The highest average RQ for the sum of the remaining OHCs (minus ΣPCBs) was estimated to be 2.3 for East Greenland of which ∑MeSO2-PCBs contributed a RQ of 1.4 (Fig. 2; Supplementary information Table S2). Only in East Greenland the RQ of the upper 95% CI was slightly above 1 (1.1) (Supplementary information Table S2). The RQ fell below 1 without the PCB and metabolite ∑$MeSO_2$–PCB contribution for all polar bear subpopulations, suggesting no likely OHC exposure risk without the contribution from PCBs. On average, among the investigated populations the OHC risk contributions declined in the order: ∑PCBs (90.5%) > ∑$MeSO_2$–PCBs (5.3%) > PFOS (1.9%) > ∑CHLs (1.2%) > ∑DDTs (0.6%) > dieldrin (0.2%) ~ β-HCH (0.2%) > ∑ClBz (0.05%) > α-HCH (0.04%) > ∑PBDEs (0.01%).

PCBs and their hydroxylated-metabolites are known to exhibit various endocrine disrupting properties such as estrogenic and thyroidogenic effects (Andersson et al., 1999; Colborn and Clement, 1992; Colborn et al., 1993; Gabrielsen et al., 2015; Kramer et al., 1997; Matthews and Zacharewski, 2000; Moore et al., 1997). Controlled laboratory studies of rodents and primates have shown that these compounds interfere with male and female reproductive performance such as testes and endometrial pathology, sperm quality, foetal development, growth and litter size at food concentrations of 8–250 μg/kg/day and adipose tissue concentrations of 7.5–77 μg/g lw (AMAP, 1998, 2004; Golub et al., 1991; WHO, 1992). Numerous laboratory studies have also shown that PCBs induce negative reproductive effects in mink (*Mustela vison*) similar to those in rats (e.g. Lund et al., 1999). Mink, however, are more highly susceptible to the toxic effects of PCBs, and it is therefore troublesome to extrapolate effect levels to Arctic species (Restum et al., 1998). Nonetheless, mink are a valuable study species as their reproductive cycle includes delayed implantation similar to that of polar bears and other marine mammals. A study

Table 2
Critical Body Residues (CBR) for reproduction (embryotoxicity, teratogenicity), immunosuppression and carcinogenicity (genotoxicity). CBR were calculated from Critical Daily Doses (CDD) of rats extracted from the literature (see Supplementary Information Table 1) and a physiologically-based pharmacokinetic (PBPK) model. #N/A: Data not available in the literature.

Contaminant	Contaminant used to modell RQ	CBR reproduction	CBR immune system	CBR carcinogenity	Unit	Tissue
∑PCB	PCB (Arochlor 1260)	469	469	469	ng/g l.w.	Blubber
∑$MeSO_2$-PCB	PCB (Arochlor 1260)	469	469	469	ng/g l.w.	Blubber
∑DDT	DDT	1806	6502	225,770	ng/g l.w.	Blubber
∑ClBz	HCB	2459	49,185	#N/A	ng/g l.w.	Blubber
Dieldrin	Dieldrin	9579	12,452	479	ng/g l.w.	Blubber
α-HCH	γ-HCH	13,024	1302	1302	ng/g l.w.	Blubber
β-HCH	γ-HCH	13,024	1302	1302	ng/g l.w.	Blubber
∑CHL	Chlordane	16,355	16,355	332,213	ng/g l.w.	Blubber
∑PBDE	BDE-99	150,601	#N/A	#N/A	ng/g l.w.	Blubber
PFOS	PFOS	4678	9356	655	ng/g w.w	Liver

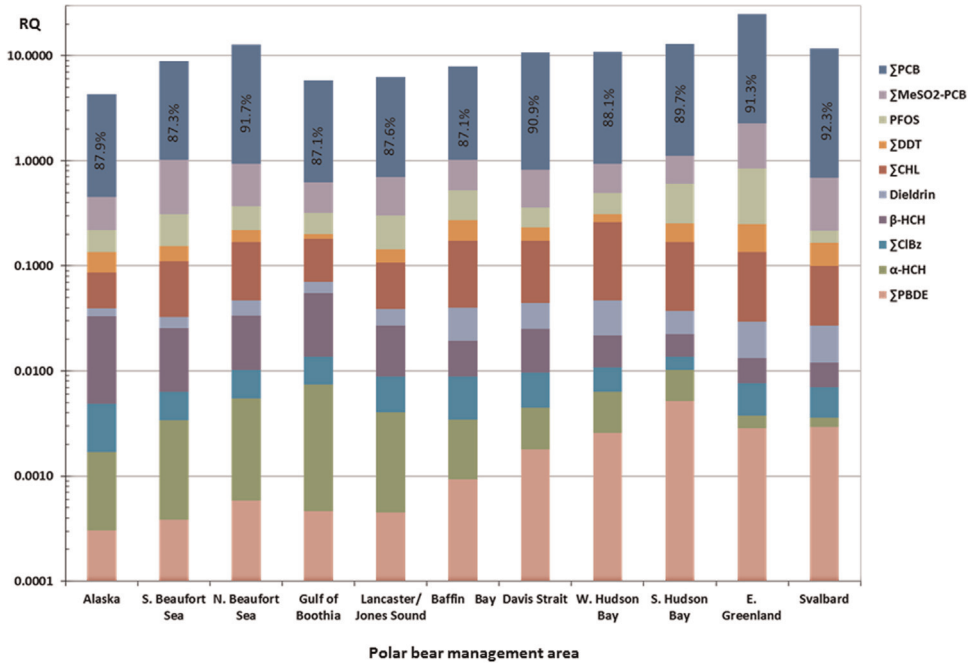

Fig. 2. Risk Quotients based on monitoring trans-Arctic POP data of polar bears from McKinney et al. (2011) and Letcher (unpublished) and estimated CBR values for effects on reproduction. The percent contribution of PCBs to the sum RQ is displayed to highlight their significance to the overall risk. Geometric mean as well as mean and max values are provided in Supplementary information Table S1. See Supplementary information Fig. S1 for plot with linear *y*-axis (non-logarithmic).

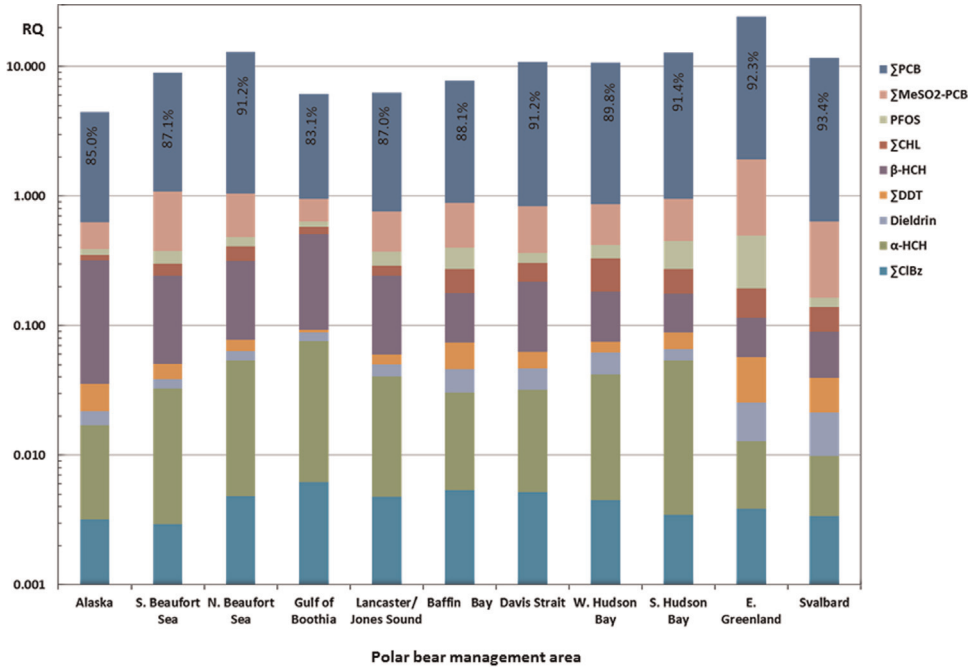

Fig. 3. Risk Quotients based on monitoring trans-Arctic POP data of polar bears from McKinney et al. (2011) and Letcher (unpublished) and estimated CBR values for effects on the immune system. The percent contribution of PCBs to the sum RQ is displayed to highlight their significance to the overall risk. Geometric mean as well as mean and max values are provided in Supplementary information Table 2. See Supplementary information Fig. S2 for plot with linear *y*-axis (non-logarithmic).

of continuous oral exposure in mink to 250 ng PCB/g food of contaminated fish induced oestrus delay and lowered whelping rates, while dietary oral exposure to a concentration of 500 ng PCB/g increased litter mortality and lesser body weights than controls (Restum et al., 1998). Another study of mink feeding on environmentally relevant concentrations of e.g. $MeSO_2$–PCB metabolites for one year showed lower birth weights and reduced kit survival at muscle concentrations of 18 and 21 μg/g lw (Lund et al., 1999). These concentrations were ca. 20 fold higher than the upper 95% confidence limits of the ∑$MeSO_2$–PCB in blubber of East Greenland bears and less than a factor 2 higher than the corresponding values for ∑PCB.

PBPK modelling on reproductive effects has previously been conducted for East Greenland polar bears over three time periods (Sonne et al., 2009). In concordance with the present study their results showed that subcutaneous adipose tissue concentrations of PCBs were in the range to elicit possible adverse health effects on reproduction (all RQ > 1) for the years 1990, 2000 and 2006. The concentrations of oxychlordane, DDTs, HCB and HCHs in polar bears resulted in RQs < 1 and thus appear less likely to be linked to

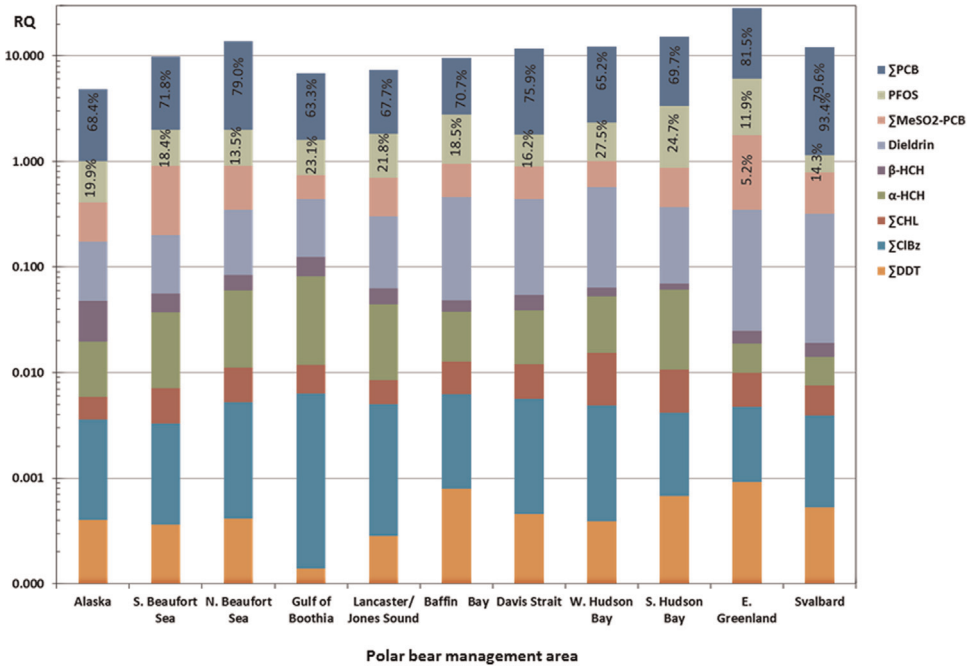

Fig. 4. Risk Quotients based on monitoring trans-Arctic POP data of polar bears from McKinney et al. (2011) and Letcher (unpublished) and estimated CBR values for carcinogenic effects. The percent contribution of PCBs and PFOS to the sum RQ is displayed to highlight their significance to the overall risk. Geometric mean as well as mean and max values are provided in Supplementary information Table 3. See Supplementary information Fig. S3 for plot with linear *y*-axis (non-logarithmic).

reproductive effects. East Greenland polar bears contain the highest blood levels of PCBs and/or OH-metabolites among all polar bear populations studied and indeed among any Arctic species (Gebbink et al., 2008; Letcher et al., 2010; McKinney et al., 2011). Because of extreme logistic and financial challenges, little research on the effect of these high PCB concentrations on reproduction and ontogenetic development has been conducted in polar bears (Letcher et al., 2010). Sonne et al. (2006a, 2007a, 2015) reported inverse correlations between ΣPCBs concentrations and size of male and female sexual organs at adipose tissue and food concentrations of 0.8–20 μg/g lw and 16 μg/kg/day, respectively, suggesting a pre- and postnatal neuro-endocrine disruption of the thalamic–hypothalamic–pituitary-target organ axis (e.g. Colborn and Clement, 1992; Colborn et al., 1993; Faroon et al., 2001; Langer, 2008; Morse et al., 1993; Tryphonas, 1994; Tung and Iqbal, 1997; Zoeller et al., 2002). These concentrations and relationships are similar to those from controlled laboratory studies and may lead to similar effects on reduced sperm / egg and baculum quality / quantity, resulting in possible lower reproductive rates and population declines (e.g. Baskin et al., 2001; Bergeron et al. 1990; Fielden et al., 2001; Hosie et al., 2000; James, 1999; Jarrell et al., 2002; Sharpe and Skakkebæk, 2003; Skakkebæk et al., 2001). A recent study actually showed a decline in baculum bone mineral density from central Canada towards East Greenland along the increasing gradient of PCBs (Sonne et al., 2015). Derocher et al. (2003) discussed how OHCs could have affected the demography of Svalbard polar bears, and as East Greenland polar bears have similar chemical body residues, similar demographic impacts may also be occurring. Nonetheless, the present findings of RQs ≥ 1 for all study regions indicates that all polar bear populations are at risk of reproductive health effects. A few East Greenland and Svalbard polar bear studies have reported on morphological changes in sexual organs (enlarged and penis-formed clitoris) that theoretically could be related to PCB exposure, but no solid conclusions were made (Sonne et al., 2005a; Wiig et al., 1998). It has been suggested by Sonne et al. (2005a) that the enlargement of polar bear clitoris observed in the wild could be part of seasonal endogenous variations in hormonal and sexual activities. If that was the case such seasonal enlargements might appear in captive polar bears as well. Another reproductive effect reported from the Arctic was a case of *hypospadia* in an East Greenland male sledge dog. Such malformations will prevent animals from reproducing themselves (Sonne et al., 2008a).

The second largest contributor to total RQ was PFOS, which only showed a RQ > 1 for the maximum value of the East Greenland bears. However, PFOS and other PFAAs have been increasing over the recent three decades until 2007 (Dietz et al., 2008; Rigét et al., 2013). In laboratory studies, the effect dose of PFOS for reducing pup survival and weight of rats was estimated at 1.6–3.2 mg/kg/day (~internal dose of 58 μg/g liver ww) while Lowest Observed Adverse Effect Level (LOAEL) was 0.4 mg/kg/day and NOAEL 0.1 mg/kg/day (~internal dose of 15 μg/g liver ww) (Seed, 2000). In rabbits, PFOS caused maternal toxicity (decreased body weight gain) at 1 mg/kg/day or higher (Case et al., 2001) and caused abortions and reduced foetal weights. Beach et al. (2006) determined population reproductive (and immune) effects at 5000 ng/g ww in the liver, which is only 2.5 times above the geometric mean and within the 95% CI PFOS of the East Greenland polar bears. In East Greenland polar bears, an increase of perfluorinated sulphonates (PFSAs; including PFOS) were observed up to 2007, where the present bears were sampled (Dietz et al., 2008; Rigét et al., 2013). Since then a decline has been observed in East Greenland, which means that the PFOS contribution was modelled during the most severe time period for this region. This may also apply to other regions as several studies have indicated a decrease in PFOS levels over time. In contrast, perfluoroalkyl carboxylate (PFCAs) concentrations have tended to increase in tissues of aquatic organisms at many locations (reviewed by Houde et al., 2011).

3.2. *Effects on the immune system*

The sum RQ for effects on the immune system were above 1 for all polar bear populations and ranged from 4.5 in Chukchi Sea/Alaska to 24.4 in East Greenland (Fig. 3). As with reproductive effects, ∑PCBs was the main contributor to the RQ on the immune

system, contributing between 83 and 93% to the total RQ and alone exceeding the RQ threshold (> 1) for all populations. The highest risk was found for East Greenland (∑PCBs RQ=22.5) and lowest for the Chukchi Sea/Alaska (∑PCBs RQ=3.8) (Fig. 3 and Supplementary information Table S3). The sum of the RQs for the remaining OHCs were below 1 for all polar bear populations except Beaufort Sea and East Greenland. The percentage contaminant risk contributions among all populations on average declined in the order: ∑PCBs (89.7%) > ∑$MeSO_2$–PCBs (5.2%) > β-HCH (2.3%) > PFOS (0.9%) > ∑CHLs (1.2%) > α-HCH (0.4%) > ∑DDTs (0.2%) > dieldrin (0.1%) > ∑ClBz (0.05%).

The immune system is a complex network of cells with diverse functions, communicating through a wide array of messenger molecules, which has evolved to protect the host from potentially pathogenic agents including viruses, bacteria, parasites, fungi, neoplastic and non-self cells (Kuby, 2012). Thus, contaminant-induced immunotoxicity may increase an animal's susceptibility to a variety of pathogens, potentially resulting in morbidity or mortality. Contaminant-induced immunotoxicity has been demonstrated in a number of marine mammal species, as well as in humans and laboratory animals, upon both in vitro and in vivo exposure. Non-coplanar PCBs, more than coplanar dioxin-like PCBs, were shown to modulate harbour seal (*Phoca vitulina*), common dolphin (*Delphinus delphis*), beluga (*Delphinapterus leucas*), and human innate immune functions (Levin et al., 2004, 2005a,b,c, 2007).

The primary studies of immunotoxicity in polar bears include Bernhoft et al. (2000) and Lie et al. (2004, 2005), which together with previous works on immune response (and retinoid and hormone levels) and OHCs in blood plasma of free-ranging Svalbard and East Greenland polar bears is summarised by Letcher et al. (2010). In their study of polar bears from Svalbard and Canada, where bears were recaptured 32–40 days after immunisation with inactivated influenza virus, reovirus, herpes virus and tetanus toxoid, Lie et al. (2004) found that PCBs negatively influenced serum immunoglobulin-G levels, increased titers against influenza virus and reovirus, and increased antibody production against tetanus toxoid. Furthermore, Lie et al. (2005) documented that high concentrations of PCBs and/or organochlorine pesticides (OCPs) correlated with reduced specific lymphocyte function and thus may produce impaired resistance against infections in Svalbard polar bears. These strong effects due to PCB exposure in the Svalbard bears are in concordance with our modelled results, where the RQ of the ΣPCB was highest in East Greenland and Svalbard but also above 1 in the other polar bear populations. Thus, as concluded in the previous AMAP assessment (de Wit et al., 2004), high OC levels may impair polar bears' ability to produce antibodies and may thus cause impaired resistance to infections.

Histopathological examination of immune organs, including lymph nodes, spleen, thymus and thyroid tissue from East Greenland polar bears (1999–2002) documented high secondary follicle count in spleen and lymph nodes, which was significantly higher in subadults compared to adults of both sexes (Kirkegaard et al., 2005). Most of the correlations between concentrations of OHCs and the amount of secondary follicles in lymph nodes were insignificant, but ΣPBDEs showed a significant and modest positive correlation. Unfortunately ΣPBDEs were not included in our model as CBRs were not available for the PBPK modelling and risk calculations. In spleen, a significant relationship between low concentrations of ΣCHLs, ΣHCHs, HCB and dieldrin in adipose tissue and few/absent secondary follicles was found. Kirkegaard et al. (2005) suggested that, based on the available sample exposure of polar bears to OHCs, it was unlikely to have resulted in adverse effects on the histopathology of the tissues in question.

The most conclusive support for a cause–effect relationship between measured OHC exposure and immunotoxicity for polar bears, as well as traditional Greenlandic peoples, came from the results of a controlled study on the closest surrogate to polar bears, domestic West Greenland sledge dogs (*Canis familiaris*) (Sonne et al., 2006b). The exposed sledge dog group was fed a diet of minke whale (*Balaenoptera acutorostrata*) blubber rich in OHCs and n-3 fatty acids and with exposure levels similar to those of polar bears and Inuit, while the control group was fed a diet of uncontaminated pork fat. The study documented that a daily intake of 50–200 g of minke whale blubber for 21–52 weeks caused an impairment of both the non-specific and specific cellular immune system in the sledge dogs. Acute phase complement protein and cytokine RNA expression was also studied in the same cohort of sledge dogs after exposure for up to ca. 100 weeks, and suppression was observed for liver haptoglobin (HP) and fatty acid binding protein (FABP) in the exposed group (Sonne et al., 2007b). These studies concluded that the combination of OHCs and marine n3/n6-fatty acids as well as microelements from the minke whale blubber had an impact on the immune status/reactions in this polar bear surrogate species, suggesting similar immunosuppression and decreased resistance to diseases in polar bears exposed to a similar marine mammal diet.

There is also increasing evidence that PFOS (and Perfluorooctanoic Acid, PFOA), having the second highest RQ but below 1 in the polar bears, modulates inflammatory responses, production of cytokines, and adaptive and innate immune responses in rodent, avian and reptile models, as well as in mammalian and non-mammalian wildlife (De Witt et al., 2012; Peden-Adams et al., 2008; Sonne, 2010). As previously mentioned Beach et al. (2006) determined population immune (and reproductive) effects at 5000 ng/g ww, which is only 2.5 times above the geometric mean and within the 95% CI PFOS of the East Greenland polar bears. Since several PFCAs/PFSAs have been shown to be immunotoxic in laboratory animals (Keil et al., 2008; Lau et al., 2007; Peden-Adams et al., 2008), but as only PFOS were included in our modelling, we are underestimating the effects of all the PFCAs/PFSAs. On the other hand this study investigated bears around the highest exposure period of PFOS (see previous section for time trend information).

3.3. Carcinogenic effect

The sum RQ for carcinogenic effects were above 1 for all polar bear populations and ranged from 5.1 in Chukchi Sea/Alaska to 28.6 in East Greenland (Fig. 4). ∑PCBs (70.6-90.2%) and then PFOS (2.9–18.7%) were the main contributors to the total RQ. The RQ of ∑PCBs were likewise all above 1 and ranged from 3.8 in Chukchi Sea/Alaska to 22.5 in East Greenland, while PFOS contributions were > 1 in seven of eleven populations ranging from 0.35 in Svalbard to 4.3 in East Greenland. When ∑PCBs and PFOS were not included, the sum of the remaining OHCs for all polar bear populations had geometric means close to 1 of which seven out of the eleven populations were > 1 (Fig. 4; Supplementary information Table S4). The contaminant risk contributions declined in the order: ∑PCBs (78.6%) > PFOS (11.7%) > ∑$MeSO_2$–PCBs (4.6%) > dieldrin (2.9%) > β-HCH (2.0%) > α-HCH (0.3%) > ∑CHLs (0.05%) > ∑ClBz (0.04%) > ∑DDTs (0.004%).

Peroxisomes are cellular organelles found primarily in the liver and kidney that contain enzymes essential for lipid metabolism, cellular respiration and gluconeogenesis among other things (Youssef and Badr, 1998). A number of OHCs, including perfluorinated compounds, are potent peroxisome proliferators, and increased oxidative stress by peroxisome proliferation is thought to be one possible mechanism for tumour promotion (Cattley and Preston, 1995). The induction of CYP enzymes by e.g. polycyclic aromatic hydrocarbons (PAHs) can also induce oxidative stress,

which may be manifested by increased production of reactive oxygen species, lipid peroxidation and DNA damage (Hennig et al., 1999; Park et al., 1996; Schlezinger et al., 1999; Slezak et al., 1999; Slim et al., 1999, Toborek et al., 1995). Down-regulation of gap junctional intercellular communication (GJIC) is suspected as another mechanism in the tumour-promoting properties of many carcinogens (Trosko and Ruch, 1998). As gap junction proteins allow for the transport of substances between cells, a process necessary for normal cell growth and function, inhibition of GJIC may lead to tumour promotion. Various PCBs, DDTs, dieldrin, toxaphene and brominated biphenyls have been shown to inhibit GJIC in human breast epithelial cells (Kang et al., 1996) and $MeSO_2$–PCBs seem particularly potent (Kato et al., 1998). The coplanar PCBs, CB77 and 169, as well as 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), inhibited GJIC in mouse Hepa1c1c7 cells (de Haan et al., 1994), and 14 PCB congeners inhibited GJIC in rat liver white blood cell culture (Andersson, 2000). In the present study we were not able to evaluate the RQ on the individual PCB congener level (and other OHC) as the geographical trends and critical body residues were not available for all individual congeners. The lack of a trans-Arctic reporting of coplanar PCBs tend to underestimate our evaluation of the RQ of the present study, whereas it is uncertain whether the lack of congener specific risk handling may over- or under-estimate the RQs.

Belugas from the St. Lawrence Estuary (SLE) are among the world's most POP contaminated wildlife populations and have therefore been studied for the past twenty years (see summary in AMAP, 2004). For instance, levels of ΣPCBs have been reported up to 86 and 45 μg/g (lw) in blubber and liver respectively (e.g., Letcher et al., 2000; Martineau et al., 1987; McKinney et al., 2006). These levels are up to 10-fold higher than in Arctic belugas (e.g. western Hudson Bay) (Andersson et al., 2001; Letcher et al., 2000; McKinney et al., 2006) as well as most polar bear populations; though, polar bears in the 1980s, and in most recent years in East Greenland, are within a factor of two of those levels (Dietz et al. 2013a, unpublished). Several reports have associated high levels of POPs with severe lesions (likely lethal), widespread infections and a high rate of neoplasia (an abnormal proliferation of cells) for the SLE beluga population (e.g., De Guise et al., 1995, 1998; Martineau et al., 1987, 1994). This rate of neoplasia is alarming as compared to other wildlife populations (Martineau et al., 2002). However, comparisons to more northerly populations are complicated by logistical constraints and variations in sampling bias (e.g. hunter-killed animals are typically healthy compared to stranded animals). Thus, a basic comparison of prevalence rates of neoplasia or other pathologies is confounded by this selection bias as well as by age and sex and other environmental factors (e.g., proximity to specific industries and municipalities). Nonetheless, stranded SLE belugas are affected by relatively high rates of cancers of the digestive tract which could be associated with exposure to the well-known mutagenic/carcinogenic polycyclic aromatic hydrocarbons (PAHs) (e.g. benzo[a]pyrene) from local aluminum smelters (Martineau et al., 2002). This type of exposure in the high Arctic is currently unlikely for marine mammal populations.

3.4. Considerations

Based on the present RQ calculations, other Arctic mammals such as the Arctic fox (*Vulpes lagopus*) and killer whale (*Orcinus orca*) and other toothed whales (AMAP 1998, 2004; Fuglei et al., 2007; Letcher et al., 2010; Wolkers et al., 2007) may be at risk due to high OHC exposure and subsequent body burdens. In addition, Inuit who consume the seals and other marine mammals from the same area as the trans-Arctic polar bears are also likely to be at risk. That was also the conclusion of a risk assessment of the Greenland Inuit population by Nielsen et al. (2006). In addition to the risk for reproductive success, immune effects and carcinogenic effects calculated in the present study, possible health effects on other key parameters such as vitamin concentrations (Braathen et al., 2004; Skaare et al., 2001), hormone concentrations (Braathen et al. 2004; Haave et al., 2003; Oskam et al., 2003, 2004; Skaare et al., 2001), CYP450 (Bandiera et al., 1995; Letcher et al., 1996), bone (Sonne et al., 2004, 2006a) and internal organs (Sonne et al., 2005b, 2006b, 2007b, 2008b) should be added in order to complete the list of possible OHC effects in polar bears.

Polar bears are a typical k-selected species (long living and low reproductive competitor species in crowded niches), which makes them vulnerable to impacts on reproduction. The present study is based on the best available data and the assumption that rat data for OHC reproductive sensitivity can be extrapolated to polar bears. However, the data stresses that dose–response effect studies should be carried out on these vital parameters and subsequently having population effect modelling carried out. The present study is calculated on trans-Arctic OHC data from 2005 to 2008, which for e.g. East Greenland is the period with the lowest OHC due to a prior decline as a consequence of international regulation; however, recent analyses suggest a post-2000 increase in OHCs possibly caused by climate-related changes in feeding ecology (Dietz et al., 2013a,b, unpublished; McKinney et al., 2013). PFOS on the other hand was modelled during a period of the highest concentrations (Dietz et al., 2008; Houde et al., 2011; Rigét et al., 2013). Whether or not the combined effects of the reproductive system, immune system and for carcinogenicity/genotoxicity will be possible to be detected on the population level was outside the scope of this investigation. Recently the IUCN Polar Bear Specialist Group came out with the most updated predictions on population trends which we summarised in Table 1 (Vongraven and York 2014). Of the 11 polar bear management areas modelled in this study population trend information was lacking from four areas (East Greenland, Svalbard, the Chucki Sea and Lancaster/Jones Sound). The populations were considered stable fom another four regions namely: the N. Beaufort Sea, Gulf of Boothia, Davis Strait and S. Hudson Bay. Three areas were however, considered to be declining namely the S. Beaufort Sea, Baffin Bay and W. Hudson Bay. It would be relevant to conduct population effects modelling of contaminant and link these to hunt as well and regional climate change effects in combined effect modelling in order to better understand these population trends.

4. Conclusions

The sum RQ for effects on the reproductive system, immune system and for carcinogenicity/genotoxicity were above the toxic threshold (>1) for all polar bear populations assessed, suggesting risk for OHC additive effects for all polar bear populations. PCBs were the main contributor for all three effect categories, contributing between 71.3% and 98.0% of the total risk. PFOS was the second highest effect contributor for the three investigated effects categories, and contributions for the carcinogenic effects were also above 1 within all regions for this compound. Thus, previous suggestions of possible adverse health effects in polar bears correlated to OHC exposure are supported by the present study. This study once more indicates that PBPK modelling is an important supportive tool in the evaluation of possible OHC-mediated health effects for Arctic wildlife. However we also suggest that Critical Daily Doses (CDD) should be investigated in "*ex vivo*" dose–response studies on polar bears to replace laboratory studies on rats (*R. rattus*) to reveal whether high RQs are maintained.

Conflict of interest

No conflicts of interest were reported.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2015.03.011.

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