Full length article

Mother-child transfer rates of organohalogen compounds up to four years of age

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\textbf{ABSTRACT}

\textbf{Background:} Breastfed children absorb persistent and toxic chemicals such as organohalogen compounds (OHCs) during the entire lactation period. Nursing is a main contributor to the burden of these pollutants in the first years of life, hence further assessments on the OHC load processes are needed.

\textbf{Objectives:} To identify the determinants of OHC increase in children at four years of age, considering concentration gains, maternal venous concentrations and breastfeeding time.

\textbf{Methods:} Concentrations of 19 organochlorine compounds (OCs) and 14 polybrominated diphenyl ethers (PBDEs) were analyzed in maternal venous (n = 466), cord blood (n = 326) and children venous serum at four years of age (n = 272) in the Asturias INMA cohort representing the Spanish general population. Data were evaluated considering the socio-demographic and individual information collected at recruitment and follow up surveys, as well as the OHC physical-chemical constants.

\textbf{Results:} The four years-old children concentration gains of the most abundant OHCs showed strong correlations ($R^2 = 0.65–0.93$) with the maternal concentrations during pregnancy and lactation period. The child gain/maternal transfer rates of most correlated pollutants were similar.

\textbf{Discussion:} Between 65 and 93\% of the variance of OCs in four years-old children was explained by the maternal concentrations during pregnancy and the lactation period. The compounds with log(Kow) $> 3.7$ (hydrophobic) showed analogous child gain/maternal transfer rates indicating similar processes of membrane lipid dissolution and passive diffusion from the epithelial cells into the milk. Molecular weight of these pollutants did not influence on these rates. Compounds with low log(Koa) such as hexachlorobenzene are more volatile and less retained, involving lower child gain/maternal transfer rates. These results may be useful to anticipate the increase of the concentrations of OCs in children using the maternal concentration of these compounds during pregnancy and the planned lactation period and to implement prophylactic measures in mothers with high venous pollutant concentrations.

1. Introduction

The burden of organohalogen compounds (OHCs) in children during the first years of age is conveyed from the mothers by transplacental delivery (Barr et al., 2005; Vizcaino et al., 2014; Wolff et al., 2007) and breastfeeding (Karmaus et al., 2001; Carrizo et al., 2006). These pollutants include organochlorines (OCs) and polybrominated diphenyl ethers (PBDEs), among others.

OCs were used intensively in agriculture and industry for several decades. They include polychlorinated biphenyls (PCBs), DDT and its metabolites, hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB) and others. Their lipophilic nature and high chemical stability lead to bioaccumulation in food chains and human tissues (Junqué et al., 2017, 2018; Bravo et al., 2019). Due to their adverse effects in humans and the environment, they were progressively banned in many countries since the 1970s and, with a few exceptions, finally banned.

PBDEs have been used as flame retardants in a wide range of products. They are semi-volatile, environmentally persistent, hydrophobic and biomagnify through the food web (Johnson-Restrepo et al., 2005). They have been found in environmental samples and human fluids and tissues (Antignac et al., 2009; Covaci and Voorspoels, 2005; Hites, 2004; Jin et al., 2009; Lunder et al., 2010; Uemura et al., 2010; Vizcaíno et al., 2011; Zhu et al., 2009), including newborns (Costa et al., 2016; Vizcaíno et al., 2011). There is public health concern for the potential human exposure to these pollutants (Sjödin et al., 2003; Birnbaum and Staskal, 2004), e.g. effects on neurodevelopment (Gascón et al., 2011). Accordingly, production and use of penta- and octa-BDE formulations were banned in the European Union in 2004. Only deca-BDE is still permitted, but the use in electronic applications was banned in Europe in 2008.

Concentrations of these pollutants have been reported in placenta, breastmilk, maternal, cord and newborn blood serum (Ando et al., 1986; Bravo et al., 2017; Carrizo et al., 2014; Llop et al., 2010; Ribas-Fitó et al., 2003; Sala et al., 2001; Vizcaíno et al., 2014). Breastfed children incorporate them during the entire lactation period as they tend to accumulate in fat (Hotham and Hotham, 2015). OHC blood concentrations are higher in breastfed than formula fed children even some years after discontinuation of breastfeeding, e.g. 3.5 years (Lanting et al., 1998), 4 years (Carrizo et al., 2006) and 7 years afterward (Karmaus et al., 2001). Children are more vulnerable to environmental pollutants than adults due to their greater exposure by high consumption of water, food and air in relation to their body weight, the immaturity and weakness of their metabolic system and longer lifetime to develop chronic diseases (Landrigan, 2016; Landrigan and Goldman, 2011). In addition, there are sensitive windows of exposure and development, in specific life stages, such as pregnancy and early childhood, in which the organism is more susceptible or vulnerable to the adverse health effects caused by exposure to environmental pollutants (Markris et al., 2008). These remarks outline the need for improving our knowledge on the main processes determining the intake of OHCs through maternal feeding within the first years of child development.

The present study is devoted to increase our understanding on the processes that determine the accumulation of OHCs in children up to four years of age. Specifically, the study is aimed to identify the influence of breastfeeding once the transplacental transfer contribution is assessed. Accordingly, the concentrations of OHCs in maternal venous serum during pregnancy (12th week),cord blood and venous blood serum at four years of age have been determined in mothers and their children (n = 272). Comparison of the maternal and children body burdens allowed us to identify the determinants of increase of these pollutants in children at four years of age, including the dependence of the concentration gain from maternal venous concentrations and breastfeeding time.

2. Methods

2.1. Study population and sampling

In 2004, the INMA Asturias cohort was established by the University of Oviedo in San Agustín Hospital (Avilés, Asturias, North-West Spain). Between 2004 and 2007, 494 pregnant women were recruited and their children were followed-up until 8 years of age (Fernández-Somoano et al., 2011; Fernández-Somoano and Tardon, 2014). Maternal serum during the 12th week of gestation (n = 466) and cord blood samples (n = 326) were collected and analysed for OHCs (Vizcaíno et al., 2014b, 2014a). The health status of these children was followed until four years of age (n = 453) and serum samples were collected (n = 272). These compounds have now been analysed in venous blood serum of the four-years-old children. Written informed consent was obtained from the parents of each child before the study, which was approved by the Asturias Regional Ethics Committee.

2.2. Analysis of persistent organic pollutants

The analyses of maternal venous, cord blood and children serum were performed with methods described elsewhere (Grimalt et al., 2010; Vizcaíno et al., 2009). Pentachlorobenzene (PeCB), hexachlorobenzene (HCB), four hexachlorocyclohexane isomers (α-, β-, δ- and γ-HCH), 4,4′-DDT, 2,4′-DDT, 2,4′-DDD, 4,4′-DDD, 2,4′-DDE and 4,4′-DDE, and seven PCB congeners (28, 52, 101, 118, 138, 153 and 180) were analysed by gas chromatography with electron capture detection (GC-ECD). Possible coelutions and structural confirmation was performed by GC coupled to mass spectrometry (GC-MS). Fourteen PBDE congeners, including deca-BDE (BDE-209), were analysed by GC-MS in negative chemical ionization mode (GC-MS-NICI). Limits of detection and quantification, LOD and LOQ, respectively, were calculated as described in Gari and Grimalt (2010). Method uncertainties calculated from repeatability were 0.3–4.5% and 0.9–9.1% of the OC and PBDE measurements, respectively (Vizcaíno et al., 2009; Grimalt et al., 2010). The methods performed satisfactorily in repeated international intercalibration exercises within the Arctic Monitoring and Assessment Program (AMAP Ring Test, 2014).

2.3. Lipid adjustment and body burden calculations

OHC concentrations were expressed in ng/ml (crude concentrations) and in ng/g lipid (lipid adjusted) using the equation described in Phillips et al. (1989). Total cholesterol and triglycerides in maternal, cord and 4-year old children samples were determined using colorimetric enzymatic methods in the General Biochemistry Laboratory of San Agustín Hospital. Total serum burdens (ng) of OHCs were estimated based on total blood volumes (ml) per weight in mothers (~65 ml/kg) and children at different times (~85 ml/kg at birth and ~75 ml/kg at 4 years) and the individual weights. The samples were processed using a Roche Diagnostics COBAS C711.

2.4. Covariates

Information on socio-demographic characteristics, parity, maternal age and pre-pregnancy weight was collected at recruitment. Time of gestation was recorded at birth and type and duration of breastfeeding was obtained in follow-up surveys. Information on children’s height and weight was gathered at 4 years post-partum visit questionnaires. Children BMI categories were based on child growth standards set by the World Health Organization (WHO, 2009), involving recommended BMI: < 16.7 kg/m² (boys) and < 16.8 kg/m² (girls), overweight: 16.7–18.2 kg/m² (boys) and 16.8–18.5 kg/m² (girls) and obesity: > 18.2 kg/m² (boys) and > 18.5 kg/m² (girls). Parity at child’s birth was categorized as no siblings, one sibling and ≥2 siblings. Duration of breastfeeding was divided in 3 categories: < 2 weeks (mainly formula-fed children), short-term (2–16 weeks) and long-term (> 16 weeks).

2.5. Data analysis

Data analysis and graphics were performed using the statistical software R (R Development Core Team, 2018). Medians and geometric means (GMs) with 95% confidence intervals (CIs) were used for descriptive analysis. Statistical differences between covariates were tested for significance using the Chi-square test. Spearman’s correlation coefficients (rho) were used to assess the correlations. Multivariate linear regression analyses were used to assess the association of socio-demographic covariates with OHC concentrations. All variables were standardized (centred at zero and scaled to two standard deviations) for inclusion in the model (Gelman, 2008). The
concentrations and breastfeeding duration were transformed into natural logarithms. The model included the following covariates: children’s sex, body mass index and breastfeeding duration, maternal age, parity at child’s birth, parity at child’s birth, breastfeeding, and maternal educational level.

This model was tested for interactions between maternal OHCs and breastfeeding duration. The final model was selected by both AIC (Akaike Information Criteria) and BIC (Bayesian Information Criteria).

### 3. Results

#### 3.1. Socio-demographic characteristics of the studied population

The characteristics of the population are described in Table 1. Forty-seven percent of the children were girls. About 64% of the children had recommended weight, 24% were overweight and 12% obese. Only 23% of the mothers were younger than 30 years, and two thirds were primiparous (61%). Concerning maternal breastfeeding, one third of the children did not received maternal breastfeeding (< 2 weeks), twenty-three percent had short breastfeeding (2–16 weeks) and almost half (48%) were breastfed for more than four months. A low percentage of mothers (16%) only attended primary school, 46% had a secondary school degree and approximately one third had a university degree. The socio-economic status encompassed a large spectrum of cases including the least affluent social class (IV-V, 56%), the middle affluent one (III; 20%) and in the most affluent levels (I-II, 24%).

#### 3.2. Organohalogen concentrations in four-year-old children

The concentrations of the most abundant OHCs in both crude and lipid-adjusted values are shown in Tables S1 and S2. 4,4′-DDE (median 64.1 ng/g lipid) was the most abundant organochlorine compound in the venous serum of the four-year-old children and was found above LOQ in all samples analysed. PCB-153, PCB-138 and HCB (medians 23.6 ng/g lipid, 20.4 ng/g lipid and 18.7 ng/g lipid, respectively), were found in more than 96% of the samples analysed. β-HCH, 4,4′-DDT, PCB-118 and PCB-180 were encountered in 70–79% of the samples and their median concentration ranged between 4.3 ng/g lipid and 14.5 ng/g lipid (Table S2). These OC concentrations were much lower than those found in four-year old children from other INMA cohorts and European populations performed some years before the current study (Gascón et al., 2015; Karlsen et al., 2017), which is consistent with a decrease in human OC levels (Jakszyn et al., 2009; Schuhmacher et al., 2009; Thomas et al., 2017).

The most abundant PBDE was BDE-209, with a median concentration of 3.1 µg/g lipid (detected above LOQ in 32% of the samples). This compound was followed by BDE-28 and BDE-99 (medians 1.7 µg/g lipid and 1.5 µg/g lipid, respectively), and found in 75–82% of the samples analysed. BDE-47 (median 0.55 µg/g lipid) was found above LOQ in 24% of the samples and BDE-153 (median 0.44 µg/g lipid) was found in a higher percentage, 42% (Table S2). These concentrations were similar to those found in previous European studies (Carrizo et al., 2007; Caspersen et al., 2016), but still lower than those found in children from the US, China or Australia recruited between 2004 and 2013 (Vuong et al., 2017; Eskenzie et al., 2013; Erkin-Cakmak et al., 2015; Xu et al., 2014; Toms et al., 2018). Higher PBDE concentrations in children than adults have been observed in some studies (e.g., Catalonia, recruited in 2002; Garí and Grimalt, 2013; Australia, recruited in 2006–2007; Toms et al., 2009) but in other sites such as Australia (recruited in 2010–2013) and Northern Quebec (recruited in 2006–2010) the trend is the opposite (Toms et al., 2018; Turgeon O’Brien et al., 2019). The current study also found similar or even higher PBDE concentrations in 4-year old children than in their mothers (Table S1).

### 4. Discussion

#### 4.1. Maternal and children body burdens of the organohalogen compounds

Body burdens of the OHC in mothers, newborns and four-year-old children have been calculated from the individual concentrations of these compounds and the weight of each cohort participant (see Methods section for details of body burden calculations). Box plots of the resulting distributions are shown in Fig. 1. As expected, the highest values correspond to the mothers and the lowest to the newborns. The occurrence of OHC in the newborns involves a transplacental transport from mother to foetus as already stated (Vizzaino et al., 2014b). The higher body burdens in four-year-old children than in newborns involves an additional intake of these pollutants in this first life period.

In some cases, the body burden increase is small, e.g. DDT, which reflects a low compound incorporation and is consistent with the low presence of this insecticide in the environment and human tissues because of the ban of the Stockholm Convention. The relatively rapid transformation of 4,4′-DDT into 4,4′-DDE and other metabolites in many environmental and biological processes also leads to a depletion of this compound in the absence of recent use.

The Stockholm convention also banned PCBs, HCHs and HCB. In Spain, the use of these OHCs was discontinued in the 80s. These restrictions likely decreased the exposure of the population to these compounds. However, human intake from environmental and diet sources still occur as observed in recent studies (Marti-Gid et al., 2010; Bosch et al., 2015; Rodriguez-Hernandez et al., 2016; Junqué et al., 2017).

### Table 1

Socio-demographic characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children included</th>
<th>Children not included</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children’s characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>127 (47)</td>
<td>104 (49)</td>
<td>0.71</td>
</tr>
<tr>
<td>Male</td>
<td>145 (53)</td>
<td>109 (51)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>Recommended weight</td>
<td>173 (64)</td>
<td>99 (71)</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>64 (24)</td>
<td>26 (18)</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>32 (12)</td>
<td>15 (11)</td>
<td></td>
</tr>
<tr>
<td>Maternal characteristics</td>
<td></td>
<td></td>
<td>0.070</td>
</tr>
<tr>
<td>Age at delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30 years</td>
<td>63 (23)</td>
<td>65 (30)</td>
<td></td>
</tr>
<tr>
<td>30–34 years</td>
<td>97 (36)</td>
<td>80 (38)</td>
<td></td>
</tr>
<tr>
<td>&gt; 35 years</td>
<td>112 (41)</td>
<td>68 (32)</td>
<td></td>
</tr>
<tr>
<td>Parity at child’s birth</td>
<td></td>
<td></td>
<td>0.023</td>
</tr>
<tr>
<td>Primiparous</td>
<td>166 (61)</td>
<td>130 (61)</td>
<td></td>
</tr>
<tr>
<td>Multiparous</td>
<td>106 (39)</td>
<td>83 (39)</td>
<td></td>
</tr>
<tr>
<td>Breastfeeding</td>
<td></td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>No (&lt; 2 weeks)</td>
<td>73 (29)</td>
<td>79 (39)</td>
<td></td>
</tr>
<tr>
<td>Short (2–16 weeks)</td>
<td>60 (23)</td>
<td>49 (24)</td>
<td></td>
</tr>
<tr>
<td>Long (&gt; 16 weeks)</td>
<td>123 (48)</td>
<td>73 (36)</td>
<td></td>
</tr>
<tr>
<td>Maternal educational level</td>
<td></td>
<td></td>
<td>0.70</td>
</tr>
<tr>
<td>Up to primary school</td>
<td>43 (16)</td>
<td>46 (21)</td>
<td></td>
</tr>
<tr>
<td>Secondary school</td>
<td>126 (46)</td>
<td>91 (41)</td>
<td></td>
</tr>
<tr>
<td>University degree</td>
<td>103 (38)</td>
<td>85 (38)</td>
<td></td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I–II (highest)</td>
<td>64 (24)</td>
<td>45 (21)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>54 (20)</td>
<td>48 (23)</td>
<td></td>
</tr>
<tr>
<td>IV–V (lowest)</td>
<td>153 (56)</td>
<td>120 (56)</td>
<td></td>
</tr>
</tbody>
</table>

Notes: 

- OHC concentrations available.
- p-value from Chi-square test between included and not included children in the OHC determination at age 4 years.

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4.2. Determinants of organohalogen compounds in children

Univariate analyses between OHC concentrations in four years-old children and several socio-demographic and life-style determinants are shown in Fig. 2. Children sex, place of residence and parity at child’s birth did not involve statistically significant concentration differences for any of the compounds analysed. In general, OHC concentrations among obese children were lower than in children with recommended weight or overweight and these differences were statistically significant for total PCBs (Fig. 2).

Breastfeeding duration was positively associated with the venous concentrations of OCs in four years-old children, while no statistically significant differences were found for PBDEs (Fig. 2). Multivariate regression models confirmed that duration of breastfeeding was the main driver of OHC in these children (Fig. 3). Maternal age was also found to be an important determinant of OHC concentrations in children, although with a minor influence (standardized beta-coefficients ranging between −0.25 and 0.25; Fig. 3). Maternal educational level and socio-economic status were found to be statistically significantly associated with total PCBs, with higher levels among children whose mothers had a university degree and belonged to the most affluent social class (Fig. 2).

One interesting feature emerging from these results is the strong similarity of the slopes of these OCs, between 0.056 and 0.062 ngchildren/(ngmother month) (Table 2). This strong uniformity is

4.3. Influence of breastfeeding on infant exposure to organohalogen compounds

The present cohort study is one of the few in which the OHC concentrations are available in maternal venous serum during pregnancy, cord blood serum and four years-old venous serum over a high number of cases (Table S1). These comprehensive data allow to evaluate the dependence of the OHC concentration gain at four years from breastfeeding transfer. This gain can be defined with Eq. (1).

\[
\text{BG}_{ij} = \frac{(C_{4ij} \times W_4 - C_{0ij} \times W_0)}{W_4}
\]

in which \(\text{BG}_{ij}\) is the concentration gain (ng/ml) of pollutant \(j\) in children \(i\); \(C_{4ij}\) and \(C_{0ij}\) are the concentrations of pollutant \(j\) (ng/ml) in children at four years and at birth, respectively; \(W_4\) and \(W_0\) are the weights (kg) of children \(i\) at four years and at birth, respectively.

The breastfeeding transfer of OHC can be related to the maternal concentrations of these compounds and the lactation time. The influence of these two parameters can be described with Eq. (2).

\[
\text{BE}_{ij} = \frac{C_{Mij} \times \text{BT}_{ij}}{W_4}
\]

in which \(\text{BE}_{ij}\) is the estimation of breastfeeding transfer (ng·month/ml) of pollutant \(j\) in children \(i\); \(C_{Mij}\) is the concentration of pollutant \(j\) in the mother of children \(i\); \(\text{BT}_{ij}\) is the breastfeeding time of children \(i\).

One interesting feature emerging from these results is the strong similarity of the slopes of these OCs, between 0.056 and 0.062 ngchildren/(ngmother month) (Table 2). This strong uniformity is

Fig. 1. Boxplot of OHC body burden (ng) in pregnant women (n = 467), at birth (cord blood, n = 326) and children at four years of age (n = 272) in the INMA Asturias birth cohort.
consistent with common transfer mechanisms and delivery rates between mother and infant during breastfeeding. Only HCB shows a significantly different lower slope, \(0.028 \text{ng children/}(\text{ng mother month})\). Examination of the physical-chemical properties of these compounds, namely the octanol-water and octanol-air coefficients, \(K_{ow}\) and \(K_{oa}\), respectively, (Table 2) shows that HCB has a log(\(K_{oa}\)) value that is significantly lower, 6.9 at 36 °C, than those of the other compounds, 8.3–9.7 (36 °C).

In contrast, the log(\(K_{ow}\)) values outline β-HCH, 3.7 at 36 °C (Table 2), from the more common range 5.4–7.1 at 36 °C of the other compounds. Hydrophobic compounds are transferred from maternal plasma to breast milk by passive diffusion and binding to lipids (Anderson and Sauberan, 2016; Hotham and Hotham, 2015; Quezada and Vafai, 2014). Kow is the main property determining water solubility and therefore the compound distribution between aqueous and organic phases. The similar slopes of β-HCH, 4,4′-DDE and PCBs indicate that compounds with log(\(K_{ow}\)) ≥ 3.7 (36 °C) are hydrophobic enough for efficient dissolution into the lipid membranes and diffusion across the cells interior as to transfer from epithelial cells into the milk.

Small molecular weight also enhances compound excretion into human milk (Sachs et al., 2019). HCB has the smallest molecular weight of the compounds showing strong correlation between organochlorine
Fig. 3. Standardized beta-coefficients from multivariate regression models for several socio-demographic characteristics on the levels of OHCs in children at 4 years of age. Models are adjusted by sex, body mass index, breastfeeding duration, parity, maternal age, socio-economic status and maternal educational level.

Fig. 4. Correlations of the concentration gain of organochlorine compounds in four years-old children (ng/ml) with the maternal transfer (concentration of these compounds during pregnancy and lactation time; ng-month/ml).
Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Kow at 25°C</th>
<th>Koa at 25°C</th>
<th>Slope</th>
<th>Intercept</th>
<th>R²</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCB</td>
<td>5.5</td>
<td>5.4</td>
<td>7.4</td>
<td>6.9</td>
<td>0.028</td>
<td>0.027 3.77 284</td>
</tr>
<tr>
<td>p-HCH</td>
<td>3.8</td>
<td>3.7</td>
<td>8.9</td>
<td>8.3</td>
<td>0.062</td>
<td>0.025 0.65 291</td>
</tr>
<tr>
<td>4,4-DDDE</td>
<td>6.0</td>
<td>5.9</td>
<td>9.3</td>
<td>8.7</td>
<td>0.061</td>
<td>0.068 0.93 318</td>
</tr>
<tr>
<td>PCB138</td>
<td>6.7</td>
<td>6.6</td>
<td>9.7</td>
<td>9.4</td>
<td>0.056</td>
<td>0.067 0.67 361</td>
</tr>
<tr>
<td>PCB153</td>
<td>6.8</td>
<td>6.6</td>
<td>9.6</td>
<td>9.1</td>
<td>0.059</td>
<td>0.067 0.79 361</td>
</tr>
<tr>
<td>PCB180</td>
<td>7.2</td>
<td>7.1</td>
<td>10.2</td>
<td>9.7</td>
<td>0.062</td>
<td>0.013 0.82 395</td>
</tr>
</tbody>
</table>

* Calculated from Schecter et al., 1989 and Beyer et al., 2002.

Declaration of Competing Interest

The authors declare they have no actual or potential competing financial interests.

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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.2019.105241.

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