Estimation of dietary intake and sources of organohalogenated contaminants among infants : 24-h duplicate diet survey in Fukuoka, Japan

Estimation of dietary intake and sources of organohalogenated contaminants among infants: 24-h duplicate diet survey in Fukuoka, Japan

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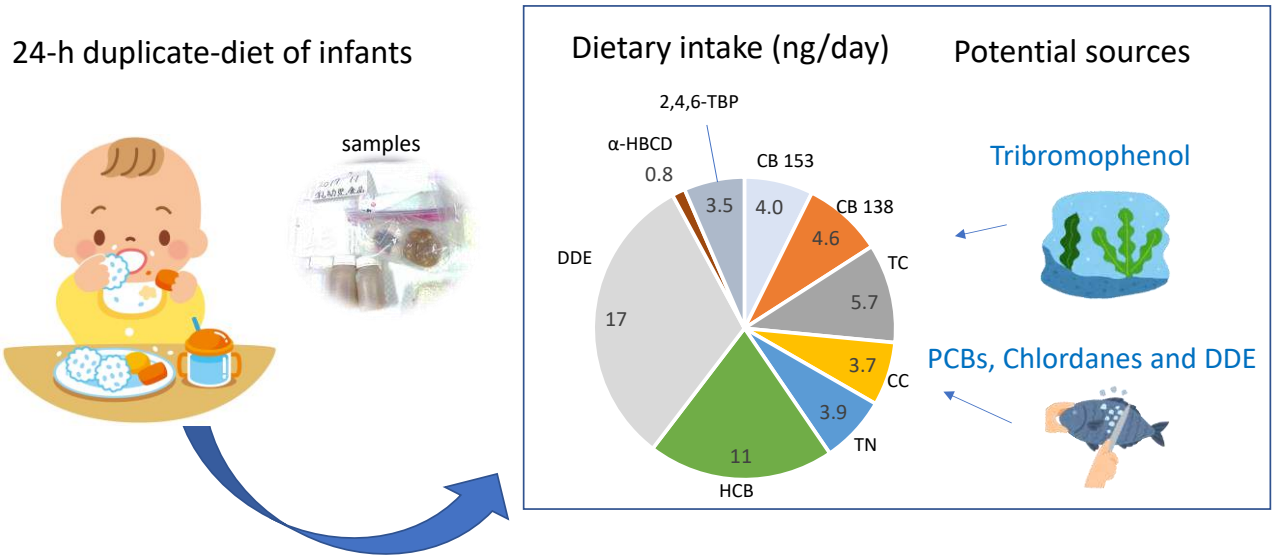
Abstract

The widespread occurrence of persistent organic pollutants (POPs) in the environment is a matter of concern. In this study, selected organohalogenated contaminants, including dichlorodiphenyltrichloroethane and its metabolites (DDTs) polychlorinated biphenyls (PCBs), chlordanes (CHLs), hexachlorobenzene (HCB), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs), tetrabromobisphenol A (TBBPA), 2,4,6-tribromophenol (TBP), were measured in complete meal sets (24-h duplicate-diet) of Japanese infants to investigate the levels, profiles, and possible sources of contamination. In total, 46 whole-day meals of infants (7 to 24-months old) were collected during 2017 from Fukuoka, Japan. To the best of our knowledge, this is the first study based on the duplicate-diet method for infants. The median intakes among the POP groups were highest for ΣDDTs (18 ng/day, maximum 251 ng/day), followed by ΣPCBs (17 ng/day, maximum 198 ng/day), ΣCHLs (14 ng/day, maximum 105 ng/day), HCB (11 ng/day, maximum 64 ng/day), TBP (3.5 ng/day, maximum 109 ng/day), ΣHBCDs (1.9 ng/day, maximum 70 ng/day), TBBPA (0.72 ng/day, maximum 34 ng/day), and ΣPBDEs (0.11 ng/day, maximum 4.5 ng/day). Among the PCBs, PCB-138 and PCB-153 were the most abundant congeners (27% and 23%, respectively). *p,p'*-DDE, the major DDT metabolite, accounted for 96% of total DDTs. Among PBDEs, BDE47 was the only detected congener (present in 4% of the samples). The dietary intake of the targeted compounds was lower than the intake via breast milk, suggesting that the exposure from baby food was limited. In the principal component analysis, chlorinated and brominated compounds were separated on principal component 1, while TBP and α-HBCD were separated on principal component 2, likely suggesting a differing emission time trend or source. PCB-153, PCB-138, trans-chlordane, cis-chlordane, and trans-nonachlor were correlated with seafood consumption (Spearman's ρ=0.45 to 0.57, $p<0.05$), while TBP was correlated with seaweed consumption (Spearman's ρ=0.46, $p<0.05$). Four species of commercial edible seaweed in Japan were analyzed to confirm the findings of the duplicate-diet study. The relatively high concentration of TBP (5.5 ± 6.6

ng/g w.w.) was observed in the seaweed samples, indicating that seaweed is a potential exposure source of TBP.

Keywords: organohalogenated compounds; duplicate-diet; baby food; Japanese infants; edible seaweed; dietary intake

49 **Graphical abstract**



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1. Introduction

The widespread occurrence of persistent organic pollutants (POPs) in the environment is a matter of great concern. Due to their stability and long-range transport properties, POPs are now widely distributed around the world and are even found in places where they have never been used, such as the arctic regions (European-Commission, 2017). Given their long half-lives and fat solubility, POPs tend to bioaccumulate in the food chain and detected in the human diet (Bramwell et al., 2016; Xu et al., 2017). Over the last decade, the priority list of POPs has been updated to include brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) (European-Commission, 2017). Despite such strict regulations, even long-banned POPs still linger in the environment; some are still in use and are directly being emitted, and new POPs may be identified for which we have limited information (European-Commission, 2017).

Brominated phenols are technically produced by bromination of phenol to contain 2, 3 or 5 bromine substituents. 2,4,6-TBP is also naturally produced in the marine environment (Gribble, 2010), and has been detected in seaweed samples in Asia (Haraguchi et al., 2010). Brominated phenols of natural origin can thus accumulate in the marine food webs. Multiple sources may explain its presence in various environmental compartments, while its presence in marine water (Vetter et al., 2009), sediments (Sim et al., 2009), invertebrates (Löfstrand et al., 2010), and fish (Chung et al., 2003; Wan et al., 2009) seems to be mainly the result of natural production.

Infants are considered a vulnerable population for chemical exposure. The younger and more immature the individual, the more different its response is from that of an adult (Bruckner, 2000). Food ingestion is a major pathway of exposure to POPs in the general population (Fraser et al., 2009). So far, however, most human monitoring studies have focused on human breast milk as an exposure pathway of halogenated compounds to Japanese infants (Fujii et al., 2011; Fujii et al., 2012b; Fujii et al., 2012c; Fujii et al., 2018; Haraguchi et al., 2009; Konishi et al., 2001; Kunisue et al., 2006; Soleman et al., 2020). Although baby food has been considered to be a potential

exposure source, reported data is still limited.

In the present study, we investigated the dietary exposure to selected classes of organohalogenated compounds among 46 Japanese infants (< 2 years old) using the duplicate-diet approach. The pollutant levels of individual food items (ng per g of food) do not necessarily reflect the daily intakes of infants, owing to the variety of available food items and cooking processes. Therefore, the dietary intake of pollutants needs to be evaluated based on the levels in complete meals consumed in 24 hours (per day per person). Also, the composition of the selected contents in the duplicate-diet samples (seafood, meat, and seaweed) was also analyzed to elucidate the possible exposure sources to organohalogenated compounds. Moreover, four species of edible seaweed from Japan (*Sargassum fusiforme, Undaria pinnatifida, Gloiopeltis, and Eisenia bicyclis)* were analyzed to confirm the findings of the duplicate-diet study.

2. Material and methods

2.1. Sample collection

2.1.1 Duplicate-diet

Baby duplicate-diet from volunteer Japanese families with children under 2 years old were used for the evaluation. Basic characteristics of the participants (sex, age in month, body weight, food consumption per day) are summarized in Table1. The overall average age of the children was 12±4 months and the body weight was 9.0±1.2 kg. This value was close to the average weight of 1year olds in the National Health and Nutrition Survey of Japan, which is 10.2 kg (MHLW, 2017). The Ethics Committee of the Daiichi University of Pharmacy approved this study (No. 17001) and appropriate written informed consent was obtained from all participants. Participants were asked to donate duplicate portions of all food and drinks consumed by their children during a 24-h period. The food self-record sheet, which was previously used for the environmental specimen bank at Kyoto University (Koizumi et al., 2009), was used with slight modifications. In this study, all the cooking was done at home by parents and the cooked food was collected by the national

105 registered dietitians and briefly confirmed to be reasonable amount and contents for each infant's
106 age, then sent to the laboratory. This method has been established as a food duplicate method and
107 can assess the exact amount of xenobiotics which the infants took (Koizumi et al., 2009;
108 Watanabe et al., 1985). A total of 46 duplicate-diet from 46 families (one duplicate-diet per
109 family) were collected as indicated in Table 1. The mean (± standard deviation (SD)) age of the
110 participant children was 12 ± 4 months.

111 Each duplicated-diet portion from every participant was separated and weighed, considering the
112 food self-record sheet, and then coded per food composition tables (Table S1) (Harada et al., 2013).
113 The food items consumed by the subjects were divided into 18 different groups. After that, the
114 duplicate-diet portions were homogenized per every participate. The final volumes were recorded,
115 and the samples were freeze-dried. The water content was determined based on the sample weight
116 after freeze-drying.

117 **2.1.2 Edible seaweed samples**

118 Since the result of the duplicate-diet study showed the correlation between intake of
119 organohalogenated compounds and seaweed consumption (see section 3.3 Correlations between
120 intake of organohalogenated compounds and food consumption), dry edible seaweed samples
121 (including *Sargassum fusiforme, Undaria pinnatifida, Gloiopeltis,* and *Eisenia bicyclis*) were
122 purchased at Japanese markets in 2018. All seaweed samples originated from Japanese coastal
123 areas. The edible portions of seaweed were homogenized for chemical analyses. A theoretical dry
124 fraction of 0.2 was used to express the concentrations on a wet weight (ww) basis.

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126 **2.2. Chemicals**

127 A total of 41 organic compounds were analyzed in this study; polychlorinated biphenyls
128 (PCBs) (20 congeners from penta-PCBs to hepta-PCBs: CB 99, 101, 105, 118, 128, 138, 146,
129 153, 156, 170, 171, 174, 177, 180, 183, 187, 196/203, 199, 206, and 209), 9 organochlorine
130 pesticides (OCs) and their metabolites (oxychlordane (OxC), trans-nonachlor (TC), cis-chlordane

(CC), trans-nonachlor (TN), cis-nonachlor (CN), hexachlorobenzene (HCB), *p,p*'-dichlorodiphenyldichloroethylene (DDE), *p,p*'-dichlorodiphenyldichloroethane (DDD) and *p,p*'-dichlorodiphenyltrichloroethane (DDT)) and 4 groups of brominated flame retardants (BFRs): PBDEs (7 congeners from tri-BDE to penta-BDE; BDE 28, 47, 99, 100, 154, 153 and 183), HBCDs (3 isomers: α-HBCD, β-HBCD, γ-HBCD), TBBPA and 2,4,6-TBP. Full names and detailed information about the targeted chemicals are listed in Table S2.

2.3. Determination of organohalogenated compounds in duplicate-diet

Analyses of the targeted compounds in duplicate-diet were performed according to previously established methods (Malarvannan et al., 2014; Poma et al., 2019), with slight modifications. Briefly, 1 g of dry sample was spiked with 50 µL of an internal standard (IS) mixture A (mixture A; CB-143: 500 pg/µL, BDE-103: 100 pg/µL, ^{13}C-HCB: 50 pg/µL), and spiked with 50 µL of an IS mixture B (mixture B; *alpha*- and *gamma*-^{13}C-HBCD: 250 pg/µL, *beta*-^{13}C-HBCD: 125 pg/µL, ^{13}C-TBBPA: 250 pg/µL, ^{13}C-TBP: 250 pg/µL) and added 5 mL of hexane:acetone (3:1, *v/v*). The tube was sonicated 5 min, then vortexed 2 min, centrifuged at 3,000 rpm for 5 min, and the supernatant was transferred to a glass tube. This procedure was repeated with 5 mL of the same clean solvent. The extract was concentrated under a gentle nitrogen stream to a volume of 0.5 mL and cleaned-up by passage onto 6 g of acidified silica (AS 44% H_2SO_4) in a 25 mL empty cartridge eluted with 20 mL of hexane and 15 mL of dichloromethane (DCM). The eluate was concentrated to 0.5 mL, and was further cleaned-up by passage onto a Silica bond elute SPE cartridge (particle size: 40 µm, compounds types; polar compounds). Agilent Technologies, Santa Clara, CA, USA) topped with 100 mg acid silica (44%), and eluted with 6 mL hex (Fraction A for PCBs, pesticides and PBDEs, followed by 12 mL of DCM (Fraction B for HBCDs, TBBPA, and TBP). Fraction A and B were concentrated to near dryness.

Fraction A was reconstituted in 50 µL of isooctane and 50 µL recovery standard (RS) (CB-207, 50 pg/µL) and further analyzed with an Agilent 6890 GC coupled to an Agilent 5973 MS operated in

electron capture negative ionization (ECNI) mode with methane gas (Agilent Technologies, Santa Clara, CA, USA). The GC system was equipped with a DB-5 capillary column (30 m x 0.25 mm x 0.25 µm). The MS was operated in selected ion monitoring (SIM) for the quantification of targeted compounds. Fraction B was reconstituted in 50 µL of MeOH and 50 µL of RS (d18-γ-HBCD, 250 pg/µL) and further analyzed with an Agilent 1290 Infinity LC system coupled to an Agilent 6460 triple Quadrupole mass spectrometer MS/MS (Agilent Technologies, Santa Clara, CA, USA). The LC system was equipped with a Luna® 3µm C18(2) 100 Å column (150 mm x 2 mm; 3 µm). The MS was operated in Multiple Reaction Monitoring (MRM) for the quantification of targeted compounds. All targeted ions are described in Malysheva et al. (2018) and Poma et al. (2019).

2.4. Quality assurance

To control for potential background contamination, six procedural blanks were run in parallel with the samples. When a compound was present in the blanks, the mean blank signal was subtracted from the sample concentration and the limits of quantification (LOQs) were defined by 3 x SD of the blank values. For compounds absent in the blanks, LOQs were based on a signal/noise ratio of 10 (S/N = 10). LOQs of the targeted compounds (ng/g wet weight) are listed in Table S2. IS recovery (average ± SD) was 82±6% for CB143 and 93±7 for BDE103. The calibration was linear and characterized by good correlation coefficients (> 0.9) for all studied compounds.

2.5. Statistical analyses

2.5.1 Duplicate-diet

Correlations among the targeted compounds and food items were tested using Spearman's rank correlation coefficient. Principal component analysis (PCA) was used to identify differences between the targeted compounds. These statistical analyses were conducted using JMP® 10 (SAS Institute Inc., Cary, NC, USA). Values below LOQ were calculated as detection frequency × LOQ (James et al., 2002). The dietary intake from the baby duplicate-diet was calculated using the

following formula: Concentration of the target compounds (ng/g w.w.) × total amount of baby food per day (g in w.w./day) (Desalegn et al., 2011; Fujii et al., 2012a; Harada et al., 2013).

2.5.2 Edible Seaweed samples

We used Monte Carlo simulations in Microsoft Excel 2016 (Redmond, WA, USA) to estimate dietary intake of TBP by multiplying food consumption of seaweed and concentrations of TBP of seaweed therein from random sampling from given distributions (Fujii et al., 2020). One thousand trials of 365 days were repeated in the following two scenarios: 1) individual consumption of seaweed was constant throughout the 365 days and TBP concentration of seaweed varied from day to day, and 2) individual consumption of seaweed and TBP concentrations were constant throughout the 365 days (conservative estimate). For scenario 1, average TBP intakes were examined for 365 days. Distributions of consumption and concentration were fitted under a normal distribution and when sampled values were negative, zero was substituted. Calculated intake was summarized to the median, 90th percentile, and 95th percentile values.

3. Results and Discussion

3.1. Organohalogenated compounds in 24-h baby duplicate diet

We examined dietary exposure to organohalogenated compounds among Japanese infants. To the best of our knowledge, this is the first study based on the duplicate-diet method for infants. A total of 46 sets of duplicate-diet samples were analyzed in this study. Among the 41 targeted compounds, 19 compounds were detected in at least one of the 46 samples (LOQ; 0.005 to 0.02 ng/g-w.w.) (Table 2, Table S2). HCB was most frequently detected (78%), followed by *p,p*'-DDE (65%), and TC (57%) (Table 2). The median intakes among the groups of compounds were highest for ΣDDTs (18 ng/day, maximum 251 ng/day), followed by ΣPCBs (17 ng/day, maximum 198 ng/day), ΣCHLs (14 ng/day, maximum 105 ng/day), HCB (11 ng/day, maximum 64 ng/day), TBP (3.5 ng/day, maximum 109 ng/day), ΣHBCDs (1.9 ng/day, maximum 70 ng/day), TBBPA (0.72 ng/day, maximum 34 ng/day), and ΣPBDEs (only BDE-47 was detected; 0.11 ng/day,

maximum 4.5 ng/day). Among PCBs, PCB-138 and PCB-153 were the most abundant congeners (27% and 23%, respectively). Among the indicator PCBs, PCB-101, 138, 153 and 180 were analysed in this study since penta to hepta PCBs were targeted. The sum of PCB-101, 138, 153 and 180 were reached 82% of ΣPCBs. *p,p*'-DDE accounted for 96% of all DDTs, suggesting that it is a reminder of the past emission. The median intakes of each individual compound were highest for *p,p'*-DDE (17 ng/day), followed by TC (5.7 ng/day), HCB (4.4 ng/day), PCB-101 (4.9 ng/day), and PCB-138 (4.6 ng/day). The brominated compounds, 2,4,6-TBP, α-HBCD, γ-HBCD, TBBPA and BDE47 were trace levels (3.5, 0.76, 0.34, 0.72 and 0.11 ng/day, respectively).

Some reports have discussed the dietary intakes of organohalogenated compounds in baby food from the US and China (Liu et al., 2014), Australia (Toms et al., 2016), Korea (Jeong et al., 2014a; Jeong et al., 2014b) and Spain (Lorán et al., 2010). These studies were not based on 24-h duplicate-diet, but rather on an individual ingredient or commercial-, homemade- baby food. The estimated dietary levels of BDE-47 in the US and China (12.9 and 5.91 ng/day; 1 to 12 months old) (Liu et al., 2014) were higher than those in Korea (0.9 ng/day; 12 months old, the body weight was assumed to be 10 kg) (Jeong et al., 2014b) and Japan (0.11 ng/day) (this study). DDT levels in Japan (18 ng/day) (this study) were comparable to previous studies from Korea (17 ng/day), while PCBs and CHLs in Japan (17 ng/day and 13 ng/day) (this study) were slightly higher than those in Korea (8 ng/day and 3 ng/day) (Jeong et al., 2014a). The highest value of PCB intake was reported from commercial baby food in Spain (38 ng/day, 12 months old) (Lorán et al., 2010). These differences may reflect the contamination pattern in those countries. Moreover, the difference of sampling method may affect the value of daily dietary intakes: These ingredient-based methods might not be completely representative of the levels of dietary intakes because they only partially reflect habitual diet and dilution effects.

According to the latest national infant nutrition survey of Japan (MHLW, 2015), weaning with baby food has started by 6 months of age in most cases (88% of total). On the other hand, weaning with baby food most often finished between 13 to 15 months of age (33.3%), and 68% of

the total had finished by 15 months of age. Thus, the food samples in this study were divided into two groups, the early stage (from 7 to 12 months old, n=26, 10 months old on average) and the late stage (from 13 to 24 months old, n=18, 16 months old on average), according to the infant habits. In the early stage, the median intakes were highest for *p,p'*-DDE (6.3 ng/day), followed by TC (4.9 ng/day), HCB (4.4 ng/day), PCB-101 (4.0 ng/day), PCB-138 (3.8 ng/day), trans-nonachlor (TN) (2.7 ng/day) and PCB-153 (2.6 ng/day). For the late stage, the intakes were highest for *p,p'*-DDE (48 ng/day), followed by HCB (21 ng/day), TC (9.8 ng/day), TN (5.5 ng/day), PCB-101 (5.4 ng/day), PCB-138 (4.8 ng/day), PCB-153 (4.6 ng/day) and CC (4.2 ng/day). The median intakes of CC, TN, HCB and α-HBCD were significantly lower in the early stage than in the late stage ($p<0.05$, t-test; $p<0.05$, Mann-Whitney U-test). Our previous research demonstrated that organohalogenated compounds in breast milk in Japan were 112 ng/g lipid for PCBs (sum of 11 isomers: PCB-74, 99, 105, 118, 138, 153, 156, 170, 180, 183, and 187), 16 ng/g lipid for HCB, 30 ng/g lipid for TN and 2.2 ng/g lipid for α-HBCD (Fujii et al., 2012b; Fujii et al., 2018). The representative value of average breast milk consumption of Japanese 10-month-old infants was reported to be 432 g/day (AIST, 2017). If we assume that the breast milk contained 3.5% of lipids, the dietary intake of organohalogenated compounds from breast milk was 2900 ng/day for PCB, 420 ng/day for HCB, 790 ng/day for TC, 58 ng/day for α-HBCD. These intake values of breastmilk were 10 to 200 times higher than those of duplicate diet in the early stage (10 months old), indicating that the intake of POPs through food consumption for Japanese infants is limited. In other words, these results indicate that breast milk is the main exposure source of POPs in infancy. However, it is important to stress that; 1) future extend study is required to confirm these results because a relatively small number of participants in this study may prevent the generalization of the findings and 2) the risk-benefit balancing approach is needed since breastmilk has the medical and neurodevelopmental advantages (Eldelman, 2012).

3.2. Correlation and principal component analysis among the targeted compounds

The nonparametric correlation coefficients among the selected targeted compounds (with DF >

30%: TC, CC, TN, HCB, *p,p*'-DDE, PCB-153, PCB-138, α-HBCD and TBP) are listed in Table S3. We found moderate to strong associations between the compounds (ρ = 0.36–0.95, $p < 0.05$) except between PCB153, PCB138 and TBP. Thus, principal component analysis (PCA) was conducted to identify clusters of the studied compounds (Fig. 1). The first and second PCA accounted for 48% and 17% of the total variance, respectively; Eigenvalues were 4.3 and 1.6, respectively. Principal component (PC) 1 had high loading matrixes on chlorinated compounds (TC, CC, TN, HCB, *p,p*'-DDE, PCB-153 and PCB-138; loading matrixes are 0.69 to 0.85), but not on brominated compounds (α-HBCD and TBP, loading matrixes are 0.30 and 0.25, respectively) (Fig. 1). On the other hand, PC2 showed a difference between TBP and α-HBCD (0.71 and -0.31, respectively). These differences may reflect the emission trend of the compounds. PC1) Time trend: the chlorinated compounds, PCBs and OCs, were already banned between 1974 to 1986 in Japan (Ministry of Justice, 1974 (last version 2018)). Therefore, the current exposure to PCBs and OCs was likely caused by past and secondary emission sources before the 1980s. On the other hand, exposure to brominated compounds (HBCDs and TBP) can be attributed to more recent emissions, as HBCDs were banned more recently (until 2010) in Japan (Ministry of Justice, 1974 (last version 2018)) and TBP is still used. PC2) Emission differences: It is known that some of the TBP is not only from artificial origins (used as a BFR or as a preservative in the timber industry) (Howe et al., 2005) but also from natural origins (occurring in marine biotas, such as marine polychaetes and fish/prawns) (Whitfield et al., 1997). Such emission differences may thus be reflected in the length and direction of the loading matrixes.

3.3. Correlations between intake of organohalogenated compounds and food consumption

The correlations between selected targeted compounds (with DF > 30%: TC, CC, TN, HCB, *p,p*'-DDE, PCB-153, PCB-138, α-HBCD, and TBP) and selected food groups (seafood (fish and shellfish), meat and seaweed) were examined for the 46 duplicate-diet samples (Table 3). Seafood and meat were used for this analysis because contamination with halogenated compounds was

previously reported in such food items (Endo et al., 2016; Nishioka et al., 2004; Teruya et al., 2002). Seaweed was also included in this analysis because naturally-occurring halogenated compounds have been detected in wild seaweed (Haraguchi et al., 2010) and these may also be present in edible seaweed.

The intake of seafood was positively correlated with the intake of PCB-153, PCB-138, CC, TC, TN (Spearman's r=0.45 to 0.57; p<0.05), while the intake of meat was positively correlated with the intake of TC, TN, HCB, *p,p'*-DDE and α-HBCD (Spearman's r =0.42 to 70; p<0.05). On the other hand, the intake of seaweed was only moderately correlated with the intake of 2,4,6-TBP (Spearman's r =0.46; p<0.05). These findings indicate that seafood and meat are potential exposure sources of PCBs, OCs and HBCDs, while seaweed is a potential source of TBP. No negative correlations were observed among the food items and the compounds.

3.4. Seaweed as a source of TBP exposure

In this study, as mentioned above, PCBs and CHLs were correlated with seafood consumption, while TBP was correlated with seaweed consumption (Table 3). It is important to note that there might be confounding factors among the food intakes and pollutant concentrations. As an example, infants who eat more meat typically also eat more grains and, as a result, the pollutants present in grains may correlate with meat intake. Seafood is reported to be the main exposure source of PCBs and chlordanes in the Japanese adult population, and meat is also an exposure source of chlordanes in some areas of Japan (Nishioka et al., 2004; Teruya et al., 2002). However, there are no previous reports about TBP and seaweed consumption. To confirm these findings, four species of edible seaweed in Japan, which were frequently consumed by the Japanese population, were analyzed. The mean concentration (±SD) of 2,4,6-TBP was 5.5±6.6 ng/g-w.w. (Table 4). We estimated TBP intake to assess the contribution of seaweed to TBP exposure in Japanese infants. The average intake of seaweed was 1.2 ± 1.9 g/day (Table S1). We conducted Monte Carlo simulations of daily intake for 365 days for 1,000 subjects, as shown in Table 4. In the first scenario, the 95th and 50th percentile values of TBP intake from seaweed were estimated to be 30.2 and 7.9 ng/day, respectively. In the

second scenario (conservative estimate), the 95th percentile value of TBP intake was higher and estimated to be 44.8 ng/day. This suggests that seaweed consumption is a potential source of TBP in the infant period.

3.5. Limitations of the study

In the current study, there are potential confounding factors among the food and pollutant intakes. In future studies, exposure estimates via seafood and meat should be confirmed by individual food analysis. Our study assumes that all consumed seaweed contains similar TBP levels to the four analyzed species. This may limit the generalization of the results. As the Japanese population consumes a wide range of seaweed and seaweed extracts, distributions by the species should also be investigated. Also, it should be noted that these findings were based on a small number of selected volunteer samples which collected only in the Fukuoka region. These features prevented to analyze the data in detailed categories such as sex, weight, district of residence. Considering these limitations, a future, more extensive study is required to further confirm these findings.

4. Conclusions

In this study, we first evaluated the dietary intakes of organohalogenated compounds, based on the levels in the complete meals of infants (per day per person). The median intakes were highest for ΣDDTs, followed by ΣPCBs, and ΣCHLs. On the other hand, the levels of BFRs were relatively lower. The dietary intake of the targeted compounds was lower than the intake via breast milk, suggesting that the exposure from baby food was limited. PCA revealed a difference between chlorinated- and brominated- compounds, suggesting a differing emission time trend or source. Several compounds were statistically correlated with the amount of seafood, meat, and seaweed consumption, indicating that the food group is a potential exposure source of organohalogenated compounds in infants. According to the individual ingredient analysis, contribution of seaweed to the total intake of TBP is discernible. More future monitoring surveys

of environmental pollutants by the complete meal sets are needed to reveal the accurate values of dietary intakes for infants.

Acknowledgments

Y.F. conceived of the study and designed the experiments. Y.F., G.P., G.M., and A.C. performed experiments and data interpretation. K.H., F.S., A.T. helped to collect the samples. A.C. supervised the research. All authors contributed to the drafting and editing of the manuscript. We thank Prof. dr. Minoru Tsuzuki and Prof. dr. Tsukasa Sakurada (Daiichi University of Pharmacy) for their support regarding the research stay of Y.F. at the University of Antwerp (UA). Financial support was provided by the Ministry of Health, Labour and Welfare of Japan (Y.F; no. H29-food-young researchers-008), and the Japan Society for the Promotion of Science (JSPS) (Y.F; no. 20K12188) and by the University of Antwerp (UA). Y.F. acknowledges a research fellowship (JSPS overseas research fellowship; no. 201860307).

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Table 1. Demographic characteristics and average food intakes of the study participants

	n	Sex	Age	Bodyweight	Food consumption	
		Male/female	(months)	(kg)	(g-ww[2)]/day)	(g-lipid/day)
Total	46[1)]	20/26	12 ± 4.0	9.0 ± 1.2	519 ± 290	11 ± 10
Early stage (7 to 12 months)	28	14/14	9.6 ± 1.7	8.6 ± 0.86	370 ± 231	5.6 ± 6.0
Late stage (13 to 24 months)	18	6/12	16 ± 3.2	9.6 ± 1.3	752 ± 207	19 ± 11

Data are presented as mean ± SD.

1) 46 duplicate-diet from 46 families (one duplicate-diet per family)

2) wet weight

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Table 2. Dietary intake of the targeted compounds from 24h duplicate-diet (ng/day)

			Total (n=46)			Early-stage (n=28) (7 to 12 months)		Late stage (n=18) (13 to 24 months)			Ma
	Compounds	n > LOQ (%)	Median	Range (Min-Max)	P95	Median	Range (Min-Max)	Median	Range (Min-Max)	t-test (two-tailed test)	h
PCBs[a]	CB 101	20	4.9	0.85 - 34	18	4.0	0.85 - 19	5.4	3.0 - 34	n.s.	
	CB 99	2	0.46	0.074 - 27	1.0	0.27	0.074 - 27	0.60	0.34 - 1.0	n.s.	
	CB 118	13	1.6	0.28 - 8.0	5.6	1.2	0.28 - 8.0	1.8	1.0 - 3.0	n.s.	
	CB 146	2	0.23	0.037 - 14	0.48	0.14	0.037 - 14	0.30	0.17 - 0.49	n.s.	
	CB 153	48	4.0	1.3 - 55	42	2.6	1.3 - 55	4.6	2.6 - 52	n.s.	
	CB 138	50	4.6	1.3 - 43	29	3.8	1.3 - 33	4.8	1.9 - 43	n.s.	
	CB 187	7	0.36	0.055 - 22	10	0.21	0.055 - 22	0.45	0.25 - 14	n.s.	
	CB 177	2	0.11	0.018 - 4.2	0.24	0.07	0.018 - 4.2	0.15	0.084 - 0.25	n.s.	
	CB 180	9	0.48	0.074 - 15	10	0.29	0.074 - 12	0.68	0.34 - 15	n.s.	
	CB 170	2	0.11	0.018 - 4.2	0.24	0.07	0.018 - 4.2	0.15	0.084 - 0.25	n.s.	
	ΣPCBs	-	17	6.2 - 198	126	14	6.2 - 198	22	11 - 161	n.s.	
Organochlorine pesticides[b]	TC	57	5.7	0.48 - 27	21	4.9	0.48 - 27	9.8	2.2 - 23	n.s.	
	CC	41	3.7	0.35 - 31	24	2.5	0.35 - 20	4.2	1.6 - 31	*	
	TN	43	3.9	0.37 - 50	36	2.7	0.37 - 20	5.5	1.7 - 50	*	
	ΣCHLs	-	14	1.2 - 105	66	11	1.2 - 60	19	5.5 - 105	*	
	HCB	78	11	0.76 - 64	42	4.4	0.76 - 32	21	9.0 - 64	*	
	p,p'-DDE	65	17	1.1 - 239	178	6.3	1.1 - 73	48	5.0 - 239	*	
	p,p'-DDT	7	0.65	0.11 - 106	30	0.41	0.11 - 1.3	1.2	0.50 - 106	n.s.	
	ΣDDTs	-	18	1.2 - 251	213	6.8	1.2 - 75	49	5.6 - 251	*	

BFRs[c]	BDE 47	4	0.11	0.018 - 4.5	2.1	0.068	0.018 - 3.2	0.15	0.084 - 4.5	n.s.
	α-HBCD	35	0.76	0.13 - 67	23	0.48	0.13 - 1.5	1.5	0.59 - 67	*
	γ-HBCD	17	0.34	0.055 - 12	6.7	0.21	0.055 - 6.1	0.55	0.25 - 12	n.s.
	ΣHBCDs	-	1.9	0.22 - 70	26	0.97	0.22 - 15	9.3	1.0 - 70	*
	TBBPA	28	0.72	0.11 - 34	9.2	0.46	0.11 - 9.0	1.0	0.50 - 34	n.s.
	2,4,6-TBP	39	3.5	0.33 - 109	100	2.1	0.33 - 94	4.1	1.9 - 109	n.s.

*$p<0.05$, significant differences between the early stage and late stage. n.s. not significant

a. CB 105, 183, 128, 174, 171, 156, 199, 196/203, 206, 209 were not detected in the all samples (Table S2)

b. OxC, CN, and *p,p*'-DDD were not detected in any sample (Table S2)

c. BDE28, 100, 99, 154, 153, 183 and β-HBCD were not detected in any sample (Table S2)

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Table 3 Spearman's rank correlation coefficients between targets compounds and food intakes

	Seafood		Meat		Seaweed	
Compounds[a]	ρ	p value	ρ	p value	ρ	p value
CB 153	**0.57**	<.0001	0.18	0.221	0.27	0.067
CB 138	**0.56**	<.0001	0.12	0.430	0.19	0.22
CC	**0.55**	<.0001	0.38	0.0089	0.30	0.044
TC	**0.45**	0.0019	**0.42**	0.0033	0.22	0.14
TN	**0.52**	0.00020	**0.45**	0.0017	0.26	0.085
HCB	0.17	0.25	**0.70**	<.0001	0.28	0.061
p,p'-DDE	0.30	0.040	**0.56**	<.0001	0.29	0.051
alpha-HBCD	0.15	0.32	**0.60**	<.0001	0.27	0.072
2,4,6-TBP	0.060	0.69	0.36	0.014	**0.46**	0.0012

a. Detection frequency of > 30% was selected (See Table 2). Bold text is used to highlight components with values more than 0.4.

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Table 4. 2,4,6-TBP concentrations in edible seaweed and the dietary intake among Japanese infants estimated by Monte Carlo simulation.

A. 2,4,6-TBP concentration in edible seaweed

Seaweed species	ng/g-w.w.
Sargassum fusiforme	10
Undaria pinnatifida	15
Gloiopeltis	0.80
Eisenia bicyclis	0.90
Average (±SD)	5.5 (± 6.6)

B. Dietary intake of TBP via seaweed consumption estimated by Monte Carlo simulation[a]

Scenario 1- individual seaweed consumption was constant throughout the year and TBP concentration varied day-by-day

	ng day^{-1}
(1) 95 percentile value	30
(2) 90 percentile value	26
(3) 50 percentile value	7.9

Scenario 2-individual seaweed consumption and TBP concentration thereof were constant throughout the year (conservative)

	ng day^{-1}
(4) 95 percentile value	45
(5) 90 percentile value	34
(6) 50 percentile value	2.8

a. Average TBP concentration in seaweed was used (5.5 ±6.6). Average consumption of seaweed was used as reported value in Table S2 (1.2±1.9). Distributions of consumption and concentration were fitted under a normal distribution and when sampled values were negative they were substituted with zero.

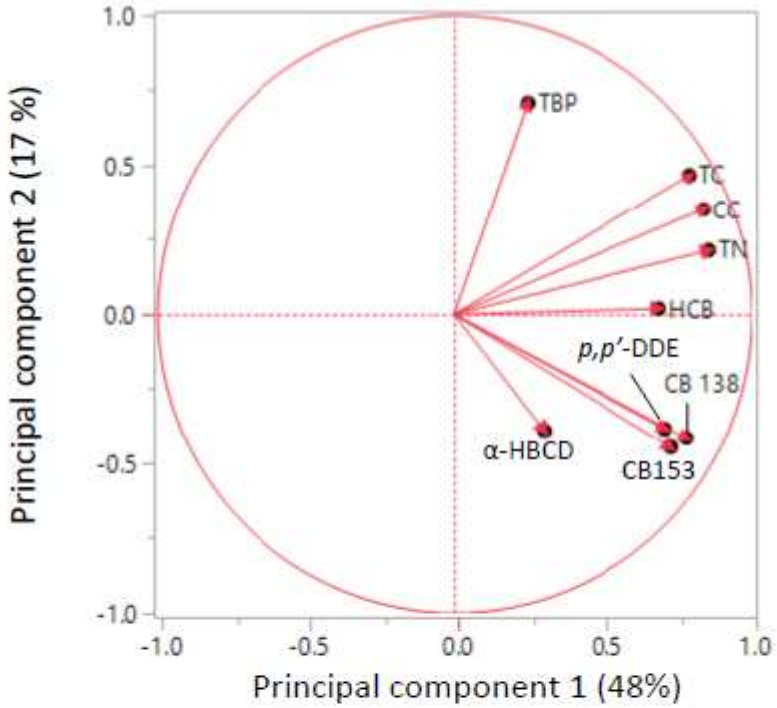

Fig.1. Principal component analysis among the target compounds 2,4,6-Tribromophenol (TBP), Trans-chlordane (TC), Cis-chlordane (CC), Trans-nonachlor (TN), Hexachlorobenzene (HCB), Dichlorodiphenyldichloroethylene (*p,p'*-DDE), 2,2',3,4,4',5'-Hexachlorobiphenyl (CB 138), 2,2',4,4',5,5'-Hexachlorobiphenyl (CB 153), alpha-Hexabromocyclododecane (α-HBCD)

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Supporting Information

Estimation of dietary intake and sources of organohalogenated contaminants among infants: 24-h duplicate diet survey in Fukuoka, Japan

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Table S1. Composition of the 24-h duplicate-diet samples

Food group	Duplicate-diet (g wet weight./day)			
	Total	Early-stage (7 to 12 months)	Late-stage (13 to 24 months)	
	n=46	*n*=28	*n*=18	
1. Rice	95 ± 57	67 ± 43	139 ± 47	*
2. Other cereals	28 ± 31	21 ± 29	39 ± 33	
3. Potatoes	11 ± 19	7.2 ± 8.8	17 ± 28	
4. Beans	15 ± 15	11 ± 12	22 ± 17	*
5. Nuts and seeds	0.022 ± 0.15	0.0±0.0	0.056 ± 0.24	
6. Vegetables	55 ± 40	48 ± 28	67 ± 53	
7. Vegetable juice	6.7 ± 25	0.50 ± 2.5	16 ± 39	*
8. Fruits	32 ± 44	13 ± 19	61 ± 54	*
9. Fruit juice	4.5 ± 20	6.0 ± 25	2.3 ± 10	
10. Mushrooms	2.9 ± 5.6	1.3 ± 2.4	5.3 ± 8.0	*
11. Seaweed	1.2 ± 1.9	0.93 ± 1.7	1.6 ± 2.3	
12. Seafood[a]	7.0 ± 8.3	6.3 ± 7.8	8.2 ± 9.2	
13. Meat	12.1 ± 11	8.3 ± 7.0	18 ± 13	*
14. Eggs	7.9 ± 17	2.1 ± 6.7	17 ± 24	*
15. Milk	80 ± 111	54 ± 98	122 ± 120	*
16. Confectioneries	8.6 ± 20	3.5 ± 8.5	16 ± 30	*
17. Beverages	97 ± 103	58 ± 72	158 ± 116	*
18. Liquids[b]	114 ± 89	107 ± 94	123 ± 83	
Water content (%)	64 ± 12	68 ± 13	59 ± 6.7	*

Data are presented as means ± SD.

*p<0.05, significant differences between the early stage and late-stage (Student's t-test)

a. fish and shellfish

b. Liquids include tap water, soup, seasonings and oils

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Table S2 Targeted compounds information

	Abbreviation	Full name	Instrument	LOQ (ng/g w.w.)	% of detected samples
PCBs	CB 101	2,2',4,5,5'-Pentachlorobiphenyl	GC-ECNI/MS	0.04	20
	CB 99	2,2',4,4',5-Pentachlorobiphenyl	GC-ECNI/MS	0.04	2
	CB 105	2,2',4,5,5'-Pentachlorobiphenyl	GC-ECNI/MS	0.04	0
	CB 118	2,2',4,4',5-Pentachlorobiphenyl	GC-ECNI/MS	0.02	13
	CB 146	2,2',3,4',5,5'- Hexachlorobiphenyl	GC-ECNI/MS	0.02	2
	CB 153	2,2',4,4',5,5'-Hexachlorobiphenyl	GC-ECNI/MS	0.01	48
	CB 138	2,2',3,4,4',5'-Hexachlorobiphenyl	GC-ECNI/MS	0.01	50
	CB 128	2,2',3,3',4,4'-Hexachlorobiphenyl	GC-ECNI/MS	0.01	7
	CB 156	2,3,3',4,4',5-Hexachlorobiphenyl	GC-ECNI/MS	0.01	0
	CB 170	2,2',3,3',4,4',5-Heptachlorobiphenyl	GC-ECNI/MS	0.01	0
	CB 171	2,2',3,3',4,4',6-Heptachlorobiphenyl	GC-ECNI/MS	0.01	0
	CB 174	2,2',3,3',4,5,6'-Heptachlorobiphenyl	GC-ECNI/MS	0.01	2
	CB 177	2,2',3,3',4,5',6'-Heptachlorobiphenyl	GC-ECNI/MS	0.01	0
	CB 180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	GC-ECNI/MS	0.01	0
	CB 183	2,2',3,4,4',5',6-Heptachlorobiphenyl	GC-ECNI/MS	0.01	9
	CB 187	2,2',3,4',5,5',6-Heptachlorobiphenyl	GC-ECNI/MS	0.01	2
	CB 199	2,2',3,3',4,5,5',6'-Octachlorobiphenyl	GC-ECNI/MS	0.01	0
		2,2',3,3',4,4',5,6'-Octachlorobiphenyl			
	CB 196/203	2,2',3,4',4,4',5,6'- Octachlorobiphenyl	GC-ECNI/MS	0.01	0
	CB 206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	GC-ECNI/MS	0.01	0
	CB 209	Decachlorobiphenyl	GC-ECNI/MS	0.01	0
Pesticides	OxC	Oxychlordane	GC-ECNI/MS	0.01	0
	TN	*Trans*-nonachlor	GC-ECNI/MS	0.01	57
	CN	*Cis*-nonachlor	GC-ECNI/MS	0.01	41
	TC	*Trans*-chlordane	GC-ECNI/MS	0.01	43
	CC	*Cis*-chlordane	GC-ECNI/MS	0.01	0
	pp-DDE	Dichlorodiphenyldichloroethylene	GC-ECNI/MS	0.02	78
	pp-DDD	Dichlorodiphenyldichloroethane	GC-ECNI/MS	0.02	65
	pp-DDT	Dichlorodiphenyltrichloroethane	GC-ECNI/MS	0.02	0
	HCB	Hexachlorobenzene	GC-ECNI/MS	0.01	7
BFRs	BDE 28	2,4,4'-Tribromodiphenyl ether	GC-ECNI/MS	0.005	0
	BDE 47	2,2',4,4'-Tetrabromodiphenyl ether	GC-ECNI/MS	0.005	0

	BDE 100	2,2',4,4',6-Pentabromodiphenyl ether	GC-ECNI/MS	0.005	0
	BDE 99	2,2',4,4'5-Pentabromodiphenyl ether	GC-ECNI/MS	0.005	0
	BDE 154	2,2',4,4',5,6'-Hexabromodiphenyl ether	GC-ECNI/MS	0.005	0
	BDE 153	2,2',4,4',5,5'-Hexabromodiphenyl ether	GC-ECNI/MS	0.005	0
	BDE 183	2,2',3,4,4',5',6-Heptabromodiphenyl ether	GC-ECNI/MS	0.005	0
	α-HBCD	alpha-Hexabromocyclododecane	LC-MS/MS	0.005	35
	β-HBCD	beta-Hexabromocyclododecane	LC-MS/MS	0.005	0
	γ-HBCD	gamma-Hexabromocyclododecane	LC-MS/MS	0.005	17
	TBBPA	Tetrabromobisphenol A	LC-MS/MS	0.005	28
	TBP	2,4,6-Tribromophenol	LC-MS/MS	0.01	39
IS	CB-143	2,2',3,4,5,6'- Hexachlorobiphenyl	GC-ECNI/MS	-	-
	BDE-103	2,2',4,5',6-Pentabromodiphenyl ether	GC-ECNI/MS	-	-
	^{13}C-HCB	^{13}C-Hexachlorobenzene	GC-ECNI/MS	-	-
	α-^{13}C-HBCD	alpha-^{13}C-Hexabromocyclododecane	LC-MS/MS	-	-
	β-^{13}C-HBCD	beta-^{13}C-Hexabromocyclododecane	LC-MS/MS	-	-
	γ-^{13}C-HBCD	gamma-^{13}C-Hexabromocyclododecane	LC-MS/MS	-	-
	^{13}C-TBBPA	^{13}C- Tetrabromobisphenol A	LC-MS/MS	-	-
	^{13}C-TBP	^{13}C-2,4,6-Tribromophenol	LC-MS/MS	-	-
RS	CB-207	2,2',3,3',4,4',5,6,6'-Nonachlorobiphenyl	GC-ECNI/MS	-	-
	d18-γ-HBCD	d18-gamma- Hexabromocyclododecane	LC-MS/MS	-	-

Chemicals was provided by Wellington Laboratories, Canada; Dr. Ehrenstorfer Laboratories, Germany, and Accustandard, USA

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Table S3. Spearman's rank correlation coefficients between the targeted compounds

Compounds[a]	CB 153	CB 138	CC	TC	TN	HCB	p,p'-DDE	α-HBCD	2,4,6-TBP
CB 153	-								
CB 138	**0.92**	-							
CC	**0.60**	**0.48**	-						
TC	**0.53**	**0.41**	**0.92**	-					
TN	**0.62**	**0.49**	**0.95**	**0.89**	-				
HCB	**0.50**	**0.36**	**0.65**	**0.64**	**0.69**	-			
p,p'-DDE	**0.71**	**0.57**	**0.63**	**0.64**	**0.69**	**0.84**	-		
α-HBCD	**0.56**	**0.42**	**0.61**	**0.56**	**0.63**	**0.78**	**0.82**	-	
2,4,6-TBP	0.27	0.21	**0.46**	**0.50**	**0.40**	**0.57**	**0.44**	**0.46**	-

Bold; $p<0.05$, significant correlation.

a. A detection frequency of 30% or more was selected.

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