JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Nonabsorbable Dietary Fat Enhances Disposal of 2,2′,4,4′-Tetrabromodiphenyl Ether in Rats through Interruption of Enterohepatic Circulation

LISETHE MEIJER,*,† ANJA M. HAFKAMP,† WOUTER E. BOSMAN,‡ RICK HAVINGA,† ÅKE BERGMAN,§ PIETER J. J. SAUER,† AND HENKJAN J. VERKADE†

Department of Pediatrics, Beatrix Children's Hospital, and Laboratory Center, Division of Binding Analysis and University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands, and Department of Environmental Chemistry, Stockholm University, SE-106 91 Stockholm, Sweden

Polybrominated diphenyl ethers are lipophilic persistent organic pollutants (POPs), which accumulate in the environment, leading to human exposure. The compounds exert a negative impact on human health. Strategies to prevent or diminish their accumulation in humans are required. We investigated in rats whether the disposal rate of ^{14}C-labeled tetrabromodiphenyl ether (BDE-47) could be enhanced by increasing fecal fat excretion through dietary treatment with nonabsorbable fat (sucrose polyester, SPE). As compared to control rats, SPE treatment increased fecal excretion rates of fat (+188%, $p < 0.05$) and ^{14}C-BDE-47 (+291%, $p < 0.05$). On the basis of biliary secretion and fecal excretion rates of ^{14}C-BDE-47, SPE effectively inhibited the enterohepatic circulation of ^{14}C-BDE-47. In conclusion, dietary supplementation of nonabsorbable fat can enhance excretion of hydrophobic POPs by interruption of their enterohepatic circulation. Our data indicate that this strategy could decrease concentrations of hydrophobic POPs in the human body and thereby their impact on human health.

KEYWORDS: BDE-47; metabolism; enterohepatic circulation; disposal; sucrose polyester

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants (POPs), which are extensively used as flame retardants in consumer products (*1*). Theoretically, up to 209 different PBDE congeners can be discriminated on the basis of position and number of bromine atoms on the phenyl rings. 2,2′,4,4′-Tetrabromodiphenyl ether (BDE-47), the main PBDE congener in the environment (*2*), is a very lipophilic compound that is virtually completely absorbed upon enteral exposure (*3*). After absorption, BDE-47 is preferentially stored in adipose tissues of animals and humans (*4*, *5*).

The physiochemical characteristics of PBDEs are rather similar to those of polychlorinated biphenyls (PCBs), which have been investigated in more detail. Exposure to PCBs in early life can occur prenatally, by transport across the placenta (*6*), and postnatally, by breast feeding. High concentrations of PCBs have been found in breast milk (*7*). Fetuses and newborns are more sensitive than adults to the toxic effects of PCBs (*8*). In children, adverse effects of PCBs have been described on neurological development (*9*), the resistance to infections (*10*), and anthropometric parameters (*11*).

The toxicity of PCBs has led to a ban on production and use of PCBs in the Western part of the world since the 1970s. However, PCBs are still present in the environment (*12*) and in humans (*13*). The production of decaBDEs has so far not been banned. Within the European Community, legislative measures regarding pentaBDE and octaBDE have been taken since 2003. Since then, it has been forbidden to produce these compounds and use these compounds in products in a concentration of more than 0.1 wt % (*14*). The prospected long-term environmental exposure of PBDEs has spurted research in strategies to enhance the disposal of these POPs from the human body.

The lipophilic character of POPs may offer the possibility to enhance their disposal from the body by stimulation of fecal fat excretion. Lipophilic compounds are hypothesized to partition into unabsorbed fat in the intestinal lumen. Stimulation of fecal fat excretion could then possibly enhance the disposal of these compounds. In 2003, we successfully stimulated fecal fat excretion in spontaneously hyperbilirubinemic Gunn rats by treatment with orlistat, a lipase inhibitor. Parallel to stimulation of fecal fat excretion, the fecal disposal of unconjugated bilirubin was increased, leading to decreased plasma concentrations (*15*). Similar to most POPs, unconjugated bilirubin is a lipophilic compound. Apart from inhibition of intestinal lipases, fecal fat excretion could be stimulated by dietary administration of sucrose polyester (SPE), a nonabsorbable fat. SPEs have been

* To whom correspondence should be addressed. Tel: 00-31-6-24671926. Fax: 00-31-50-3611704. E-mail: Lisethe.meijer@gmail.com.
† Beatrix Children's Hospital.
‡ Laboratory Center.
§ Stockholm University.

10.1021/jf0608827 CCC: $33.50
Published on Web 07/28/2006

demonstrated to enhance the disposal of various POPs in humans (*16*, *17*).

In the present study, we exposed rats to radioactively labeled BDE-47. Fecal ^{14}C-BDE-47 excretion was compared between control rats and rats treated with SPE. Balance studies and quantization of the amount of ^{14}C-BDE-47 secreted in bile allowed estimation of the effects of the treatments on relevant flux rates of BDE-47 within the enterohepatic circulation. BDE-47 was chosen as a model POP compound because of its high concentration in the environment and subsequent human exposure and its lack of appreciable metabolic conversion in rats.

MATERIALS AND METHODS

Animals. Male Wistar rats [body weight (BW), 320−405 g; Harlan, The Netherlands] were individually housed in an environmentally controlled facility with a 12−12 h light/dark cycle (7:00 AM on, 7:00 PM off) and ad libitum access to food and water. The experimental protocol was approved by the Ethics Committee for Animal Experiments, Faculty of Medical Sciences, University Medical Center Groningen (The Netherlands).

Materials and Diets. ^{14}C-BDE-47 (1.0 mCi/mmol) was synthesized from [U-^{14}C]phenol as described elsewhere (*18*). Liquid SPE, containing predominantly unsaturated long chain fatty acids, was a generous gift from Dr. J. Westrate, Unilever Research Laboratories (Vlaardingen, The Netherlands). The diets used in the study were custom-synthesized by Hope Farms BV (Woerden, The Netherlands). A semisynthetic, purified high-fat diet (16 wt % and 35 energy % fat) was used as the control diet (code 4141.07). The high-fat character was chosen for comparability to human dietary fat intake (Western human diet, ∼40 energy % fat). The standard rat diet contained approximately 15 energy % fat. In the SPE diet, 25% of the fat content of the high-fat diet (i.e., 4 wt %) was replaced by a liquid SPE, resulting in a 16 wt % fat diet, of which 12 wt % was absorbable fat (custom synthesis by Hope Farms). In previous studies, we have shown that rats tolerate high-fat diets very well (*15*).

Experimental Protocol. In a pilot experiment, intragastric administration of ^{14}C-BDE-47 to rats resulted in an absorption efficacy above 95% and in stable fecal excretion rate of ^{14}C-label from 3 days after its administration (data not shown). Only minute amounts of ^{14}C-radioactivity could be recovered from urine, and urinary collection was therefore not included in the actual experiment. On the basis of the results of the pilot study, rats were fed the high-fat (control) diet for 3 weeks, to allow adjustment to the diet. Four days before starting the experimental diets, ^{14}C-BDE-47 (dose, 15 μCi/kg BW; specific activity, 1 Ci/mol) dissolved in 0.2 mL of olive oil was administered via gastric gavage. The time period of 4 days was taken to selectively investigate the effects of SPE treatment on ^{14}C-BDE-47 wash-out. Equilibrium in intestinal uptake and disposal of the administered bolus were previously demonstrated to be present after 3−4 days in a rat study with ^{14}C-BDE-47 (*3*). These data were confirmed in our present study: Fecal ^{14}C excretion was stable during the experimental period in the control group.

At day 0, the animals were randomly assigned to continue with the high-fat diet (controls) or to receive the SPE diet for 3 weeks. Each diet group consisted of four male Wistar rats. Group sizes were determined by the (limited) availability of specially synthesized ^{14}C-BDE-47. During the following 3 weeks, feces was quantitatively collected on a daily basis, freeze-dried, homogenized, and stored at −20 °C until analysis. At days 0, 7, and 14 and the last day of the experiment (day 22−24), 1 mL of blood was obtained in heparin-coated capillaries through tail bleeding under isoflurane anesthesia. Plasma was obtained from the blood samples by centrifugation at 4000 rpm for 10 min and frozen at −20 °C until analysis. After 3 weeks on the diets, a catheter was inserted in the bile duct under intraperitoneal pentobarbital anesthesia (*19*) after which bile was collected for 30 min. Subsequently, the animals were sacrificed by collecting a large (∼10 mL), heparinized blood sample by vena cava inferior punction. Liver, testes, abdominal adipose tissue, brain, adrenal glands, and thyroid gland were collected and stored in acetone prewashed glass tubes at −20 °C until analysis.

Determination of Fecal Fat Content. The fecal fat was determined by gas chromatography of fatty acid methyl esters (HP Ultra 1 column, Hewlett-Packard, Palo Alto, CA), according to the method of Lepage and Roy (*20*), with the minor modification that instead of methanol/benzene, methanol/hexane was used for methylation and extraction. Heptadecanoic acid (C17:0) was used as an internal standard.

Determination of ^{14}C-BDE-47. To decolorize the feces, 0.5 g samples were incubated with 2 mL of bleach in a water bath (37 °C, 30 min, and mild shaking). To neutralize the free chloride, 200 μL of ammonium was added and the samples were vortexed. The samples were dissolved in 15 mL of Ultima Gold XR (Perkin-Elmer Life Science, Groningen, The Netherlands) scintillation fluid, and scintillation was measured for 10 min in a liquid scintillation counter (Tricarb 2500 scintillation counter, Perkin-Elmer Life Science). The ^{14}C-radioactivity excreted via the feces was determined by subjecting fecal samples obtained at days 0, 7, and 14 and the last day of experiment to lipid extraction according to Bligh and Dyer (*21*). Radioactivity in plasma samples was determined after adding 2 mL of Ultima Gold XR (Perkin-Elmer Life Sciences) scintillation fluid to 100 μL of plasma. Bile samples (100 μL) were first decolorized with 50 μL of bleach and dechlorinized with 15 μL of ammonium. Two milliliters of scintillation fluid was added, and scintillation was measured in the scintillation counter for 10 min. Tissue samples (100 mg) were incubated with 0.5 mL of soluene-350 in a 37 °C water bath for 48 h to allow the tissues to dissolve. Then, 0.5 mL of distilled water and 5 mL of scintillation fluid were added and the samples were stirred until clarity. Scintillation was measured in the scintillation counter for 10 min.

Determination of Enterohepatic Circulation of ^{14}C-BDE-47. The enterohepatic circulation involved hepatic secretion of ^{14}C-BDE-47 into the bile and subsequent passage into the intestinal lumen. In the intestinal lumen, ^{14}C-BDE-47 can either be (re)absorbed across the intestinal wall into the blood (i.e., the enterohepatic circulation) or excreted via the feces. We assumed that the biliary secretion rate measured during 30 min reflected 1/48th of the amount secreted per day. Fecal ^{14}C-BDE-47 was determined in 1 g of the total amount of feces in the same 24 h and multiplied by the total amount of feces in that time period. The difference between the fecal and the biliairy secretion rates per 24 h constituted the net transepithelial flux of ^{14}C-BDE-47 per day.

Statistical Analyses. All values are presented as medians with ranges. The Spearman signed rank test was used for statistical correlation. The (nonparametric) Mann−Whitney test was used for statistical comparison. Differences were considered statistically significant at $p < 0.05$. All analyses were performed in SPSS 11.0 for Windows (Chicago, IL).

RESULTS

The mean food intake during the experiment was similar in the two diet groups (per rat, ∼22 g/day). BW, feces production, and fecal fat excretion over the 3 week period are presented in **Table 1**. BW at the start and during the study was comparable between the two diet groups. SPE-treated rats had significant higher feces production rate at day 14 as compared with control rats ($p < 0.05$). Fecal fat excretion was significantly higher in the SPE group at days 7 (+223%) and 14 (+210%) and the end of the experiment (days 22−24; +188%) of treatment, as compared with the control group ($p < 0.05$).

The first day after administration of ^{14}C-BDE-47 (i.e., at 3 days before starting the experimental diets), median fecal recovery of ^{14}C-label was 2.3% of the administered dose/day (range 1.7−11.2), after which fecal excretion stabilized within days at a median of ∼0.3% of administered dose/day (range 0.1−2.0). No statistical difference in fecal radioactivity excretion was observed between groups from days 2−4. In control rats, the difference was 0.3% of the administered dose/day (range 0.3−0.4), and in SPE-treated rats, the difference was 0.4% of the administered dose/day (range 0.4−0.7), $p > 0.05$. Over the

Table 1. Body Weight, Feces Production, and Fecal Fat Excretion in Control and SPE-Treated Group over the 3 Week Experimental Period, with Statistical Difference Between the Groups[a]

	days	control group	SPE-treated group
BW (g)	0	394 (365–422)	376 (363–400)
	7	406 (368–422)	401 (378–414)
	14	431 (391–455)	402 (396–409)
	last day	440 (405–475)	421 (405–440)
feces production (g)	0	2.5 (1.9–3.1)	2.5 (2.4–2.5)
	7	2.5 (2.3–3.2)	3.1 (2.9–3.4)
	14	2.5 (2.3–2.9)	3.1 (3.1–3.2)*
	last day	2.8 (1.9–4.0)	3.7 (2.8–4.0)
fecal fat excretion	0	0.964 (0.513–1.658)	0.891 (0.686–1.018)
(mmol FFA/g feces)	7	0.896 (0.603–1.728)	2.000 (1.162–2.128)*
	14	0.946 (0.566–1.514)	1.983 (1.858–2.134)*
	last day	1.136 (0.774–1.500)	2.140 (1.806–2.524)*

[a] Data are medians, and ranges are between parentheses. $N = 4$ per diet group, and $*p < 0.05$.

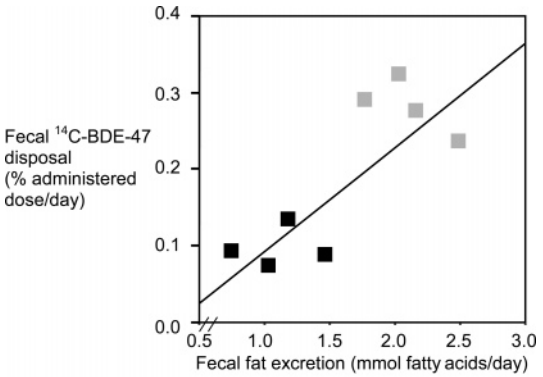

Figure 1. Relationship between fecal excretion of radioactivity from ^{14}C-BDE-47 and fat in rats treated with a high-fat diet (controls) or with a diet containing nonabsorbable SPEs. $R = 0.667$, and $p = 0.07$. Black squares, control group; gray squares, SPE group.

following 3 weeks, treatment with SPE increased ^{14}C excretion (0.4% of administered dose/day, range 0.1−0.7, $p < 0.05$) as compared with controls (0.1% of administered dose/day, range 0.1−0.4). Cumulative fecal radioactivity disposal during the 3 weeks of experimental diets was 6.9% (range 5.9−7.3) of administered dose in controls and almost 2-fold higher (13.5%, range 12.6−21.5) in SPE-treated rats ($p < 0.05$).

Figure 1 shows a trend toward positive correlation between the amount of radioactivity recovered from the feces with the amount of fecal fat excreted ($R = 0.667$, and $p = 0.07$).

Table 2 shows the effect of SPE treatment on the amount of ^{14}C-BDE-47 recovered from specific organs, tissues, plasma, and bile as compared to control diet, expressed per gram tissue or mL fluid. In both diet groups, the highest concentration of radioactivity could be recovered in adipose tissue. Interestingly, the adrenal gland and thyroid gland also contained high concentrations of radioactivity. The amount of recovered radioactivity in the different organs and tissues was comparable between the two treatment groups, except in the liver. Hepatic concentration of ^{14}C-BDE-47 per gram tissue in SPE-treated rats was lower as compared to control rats ($p < 0.05$). The liver size was comparable between the two groups (control 16.3 g, SPE 15.1 g, $p > 0.05$). Accordingly, the total amount of radioactivity present in the liver of SPE-treated rats was significantly lower ($p < 0.05$) as compared with the control group.

In both groups, the plasma radioactivity concentration was low and did not differ significantly between the groups. Mean

Table 2. Effect of SPE Treatment on the Amount of ^{14}C-BDE-47 Recovered from Specific Organs, Tissues, Plasma, and Bile, after the 3 Week Experimental Period, with Statistical Difference between the Control Group and the SPE-Treated Group[a]

	control group	SPE group	*P*
liver	0.051 (0.042–0.055)	0.035 (0.031–0.042)	<0.05
testes	0.007 (0.006–0.009)	0.008 (0.006–0.009)	Ns
adipose tissue	1.311 (1.102–1.661)	1.413 (1.057–1.775)	Ns
brain	0.009 (0.008–0.010)	0.010 (0.007–0.012)	Ns
adrenal gland	0.136 (0.018–0.152)	0.095 (0.049–0.120)	Ns
thyroid gland	0.058 (0.042–0.083)	0.048 (0.042–0.078)	Ns
plasma	0.004 (0.003–0.006)	0.004 (0.004–0.005)	Ns
bile	0.011 (0.004–0.017)	0.008 (0.004–0.008)	Ns

[a] Radioactivity expressed in % of administered dose per g tissue or per mL plasma or bile. Data are medians, and ranges are between parentheses. $N = 4$ per diet group.

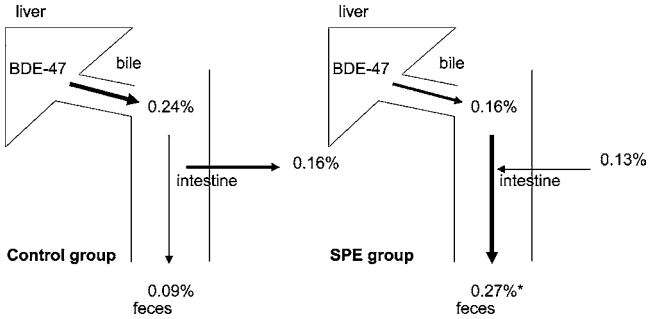

Figure 2. Enterohepatic circulation of ^{14}C-BDE-47 in the control group and SPE-treated group on the last day of the experiment. Data are median radioactivity excretion per diet group in % of administered dose/day. $N = 4$ per diet group, and $*p < 0.05$.

plasma radioactivity concentrations for all animals together were 0.011% of administered dose/mL plasma at day 0 (range 0.007−0.017), 0.006%/mL plasma at day 7 (range 0.004−0.008), 0.007%/mL plasma at day 14 (range 0.004−0.008), and 0.004%/mL plasma at the last day of the experiment (range 0.003−0.005).

Bile flow was similar in the two groups: 3.8 μL/min/100 g BW in the control group (range 3.2−4.6) and 3.6 μL/min/100 g BW in the SPE group (range 3.4−4.3). No significant difference could be observed in radioactivity excretion in bile between the control and the SPE groups.

Figure 2 shows the determined and estimated fluxes of ^{14}C-BDE-47 in the enterohepatic circulation. In the control rat, 0.24% of administered dose/day (range 0.09−0.36) was secreted with the bile, and 0.09% of administered dose/day (range 0.08−0.14) was excreted via the feces, implying an enterohepatic circulation flux of −0.16% of administered dose/day (range −0.28 to +0.04). In the SPE-treated rat, 0.16% of administered dose/day (range 0.08−0.18) was secreted with the bile, and 0.27% of administered dose/day (range 0.09−0.32) was excreted via the feces, implying an enterohepatic circulation flux of +0.13% of administered dose/day (range −0.06 to +0.17). A negative amount implies an inward flux across the intestinal epithelium into the bloodstream (enterohepatic circulation), and a positive amount implies an outward flux from the bloodstream into the intestinal lumen. The majority of ^{14}C-BDE-47 secreted in the bile of control treated rats appeared to be reabsorbed from the intestine (66%). During SPE treatment, however, the amount of ^{14}C-BDE-47 disposed of with the feces exceeded the biliary excretion rate, implying induction of net outward BDE flux across the intestinal epithelium into the intestinal lumen. This observation indicated an interruption

of the enterohepatic circulation of BDE-47 by adding SPE (nonabsorbable fat) to the diet, leading to enhanced disposal of BDE-47 with the feces.

DISCUSSION

Our results clearly indicate that stimulation of fecal fat excretion by SPE treatment enhances the fecal disposal of BDE-47. Present data on biliary secretion and fecal disposal of BDE-47 support the concept that SPE treatment interrupts the enterohepatic circulation of BDE-47 and induces a net flux of BDE-47 across the intestinal wall into the intestinal lumen.

To enhance fecal fat excretion, rats were treated with SPE (nonabsorbable fat) supplemented to their diet. SPEs are composed of sucrose molecules to which 6−8 long chain fatty acids are esterified. The acyl carboxylic ester bonds in SPEs are resistant to intestinal lipases, due to spheric hindrance. The physiochemical properties of SPEs are similar to those of triglycerides (*22*). We reasoned that treatment with SPE guarantees the permanent presence of a lipophilic phase in the intestinal lumen, which would allow association with hydrophobic compounds like BDE-47. The partition coefficient of BDE-47, reflecting the equilibrium distribution of a compound between octanol and water, is above 10 (*23*), indicating solubility in lipophilic solvents, but its virtual insolubility in aqueous solvents. SPE treatment increased both fecal fat and BDE-47 excretion. The amount of BDE-47 excreted via the feces was higher in rats with increased amounts of fecal fat excreted (**Figure 1**), supporting the hypothesis that induction of fecal fat excretion enhanced the disposal rate of BDE-47 from the body.

The present study is the first of its kind in which the effect of enhanced fecal fat excretion on the enterohepatic circulation of a specific POP is quantitatively addressed. Many lipophilic compounds are secreted into the bile from the liver and are partly reabsorbed from the intestinal lumen into the blood circulation back to the liver and, thus, undergo enterohepatic circulation. The net quantitative flux of such a compound across the intestinal wall can be estimated from the disposal rate of a compound via the feces and its biliary secretion rate. In the control group, the net quantitative flux was a "net absorption flux" (directed from the lumen into the body). In the SPE group, however, the quantitative flux was a "net excretion flux" (directed from the body into the intestinal lumen). The interpretation that SPE interrupts the enterohepatic circulation is supported by the hepatic ^{14}C-BDE-47 concentration. Part of the amount of radioactivity present in the liver is secreted via the bile into the intestinal lumen. Hepatic ^{14}C-BDE-47 content was significantly lower in SPE-treated animals than in the controls. On the basis of the important role of the biliary secretion for BDE-47 disposal, the liver is most likely the first organ in which the SPE-enhanced disposal of BDE-47 has a "wash-out" effect. We speculate that a prolonged SPE treatment would eventually result in drainage of BDE-47 from the other organs.

In this study, we found the highest concentration of BDE-47 radioactivity in adipose tissue, in accordance with previous observations (*3*). The relatively high concentration of radioactivity in the adrenal glands could be related to the high fat content of the adrenal glands (because of their production of lipophilic steroid hormones). The relatively high concentration of radioactivity in the thyroid gland could perhaps be related to binding of BDE-47 to thyroid hormone receptors present in the gland. POPs, like PBDE, influence the metabolism of the thyroid hormones by binding to the thyroid hormone receptor (*24*). The distribution of BDE-47 over the various organs is comparable with results obtained previously by Örn et al. (*3*).

In summary, SPE treatment in rats enhances fecal fat excretion and in parallel fecal disposal of BDE-47. Present data support the concept that the BDE-47 in the feces of SPE-treated rats originates from biliary secretion and also from a net flux over the intestinal wall into the intestinal lumen. In this way, SPE interrupts the enterohepatic circulation of BDE-47. The present animal model does not allow direct extrapolation of this strategy to the human situation of acute toxic or chronic environmental exposure to BDE-47 or other POPs. However, the present results justify the study of the approach in humans, also taking into account the possible side effects of SPE treatment. If applicable to the human situation, SPE treatment might decrease the exposure concentration of BDE-47, and most likely also of other POPs, thus diminishing their impact on human health.

ABBREVIATIONS USED

POPs, persistent organic pollutants; BDE-47, tetrabromodiphenyl ether; SPE, sucrose polyester; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; Wt%, weight %; BW, body weight.

LITERATURE CITED

(1) Alaee, M.; Arias, P.; Sjodin, A.; Bergman, A. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ. Int.* **2003**, *29* (6), 683−689.

(2) de Wit, C. A. An overview of brominated flame retardants in the environment. *Chemosphere* **2002**, *46* (5), 583−624.

(3) Orn, U.; Klasson-Wehler, E. Metabolism of 2,2′,4,4′-tetrabromodiphenyl ether in rat and mouse. *Xenobiotica* **1998**, *28* (2), 199−211.

(4) Meironyte Guvenius, D.; Bergman, A.; Noren, K. Polybrominated diphenyl ethers in Swedish human liver and adipose tissue. *Arch. Environ. Contam. Toxicol.* **2001**, *40* (4), 564−570.

(5) Smeds, A.; Saukko, P. Brominated flame retardants and phenolic endocrine disrupters in Finnish human adipose tissue. *Chemosphere* **2003**, *53* (9), 1123−1130.

(6) Covaci, A.; Jorens, P.; Jacquemyn, Y.; Schepens, P. Distribution of PCBs and organochlorine pesticides in umbilical cord and maternal serum. *Sci. Total Environ.* **2002**, *298* (1−3), 45−53.

(7) Jacobson, J. L.; Fein, G. G.; Jacobson, S. W.; Schwartz, P. M.; Dowler, J. K. The transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) across the human placenta and into maternal milk. *Am. J. Public Health* **1984**, *74* (4), 378−379.

(8) Guo, Y. L.; Lambert, G. H.; Hsu, C. C. Growth abnormalities in the population exposed in utero and early postnatally to polychlorinated biphenyls and dibenzofurans. *Environ. Health Perspect.* **1995**, *103* (Suppl. 6), 117−122.

(9) Koopman-Esseboom, C.; Huisman, M.; Touwen, B. C.; Boersma, E. R.; Brouwer, A.; Sauer, P. J.; Weisglas-Kuperus, N. Newborn infants diagnosed as neurologically abnormal with relation to PCB and dioxin exposure and their thyroid-hormone status. *Dev. Med. Child Neurol.* **1997**, *39* (11), 785.

(10) Weisglas-Kuperus, N.; Patandin, S.; Berbers, G. A. M.; Sas, T. C. J.; Mulder, P. G. H.; Sauer, P. J. J.; Hooijkaas, H. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. *Environ. Health Perspect.* **2000**, *108* (12), 1203−1207.

(11) Patandin, S.; Koopman-Esseboom, C.; De Ridder, M. A. J.; Weisglas-Kuperus, N.; Sauer, P. J. J. Effects of environmental exposure to polychlorinated biphenyls and dioxins on birth size and growth in Dutch children. *Pediatr. Res.* **1998**, *44* (4), 538−545.

(12) de Boer, J.; van der Zande, T. E.; Pieters, H.; Ariese, F.; Schipper, C. A.; van Brummelen, T.; Vethaak, A. D. Organic contaminants and trace metals in flounder liver and sediment from the Amsterdam and Rotterdam harbours and off the Dutch coast. *J. Environ. Monit.* **2001**, *3* (4), 386−393.

(13) Guvenius, D. M.; Aronsson, A.; Ekman-Ordeberg, G.; Bergman, A.; Noren, K. Human prenatal and postnatal exposure to polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorobiphenylols, and pentachlorophenol. *Environ. Health Perspect.* **2003**, *111* (9), 1235−1241.

(14) Cox, P.; Efthymiou, P. Directive 2003/11/EC of the European parliament and of the council of February 6 2003 amending for the 24th time Council Directive 76/669/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether, octabromodiphenyl ether). *Off. J. Eur. Union* **2003**, *OJ L* (42), 45−46.

(15) Nishioka, T.; Hafkamp, A. M.; Havinga, R.; vn Lierop, P. P.; Velvis, H.; Verkade, H. J. Orlistat treatment increases fecal bilirubin excretion and decreases plasma bilirubin concentrations in hyperbilirubinemic Gunn rats. *J. Pediatr.* **2003**, *143* (3), 327−334.

(16) Geusau, A.; Tschachler, E.; Meixner, M.; Sandermann, S.; Papke, O.; Wolf, C.; Valic, E.; Stingl, G.; McLachlan, M. Olestra increases faecal excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Lancet* **1999**, *354* (9186), 1266−1267.

(17) Moser, G. A.; McLachlan, M. S. A non-absorbable dietary fat substitute enhances elimination of persistent lipophilic contaminants in humans. *Chemosphere* **1999**, *39* (9), 1513−1521.

(18) Orn, U.; Eriksson, L.; Jakobsson, E.; Bergman, A. Synthesis and characterization of polybrominated diphenyl ethers—unlabelled and radiolabelled tetra-, penta- and hexa-bromodiphenyl ethers. *Acta Chem. Scand.* **1996**, *50*, 802−807.

(19) Kuipers, F.; Havinga, R.; Bosschieter, H.; Toorop, G. P.; Hindriks, F. R.; Vonk, R. J. Enterohepatic circulation in the rat. *Gastroenterology* **1985**, *88* (2), 403−411.

(20) Lepage, G.; Roy, C. C. Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid Res.* **1986**, *27* (1), 114−120.

(21) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37* (8), 911−917.

(22) Jandacek, R. J.; Webb, M. R. Physical properties of pure sucrose octaesters. *Chem. Phys. Lipids* **1978**, *22*, 163−176.

(23) Harner, T. Measurements of octanol-air partition coefficient (KOA) for brominated diphenyl ethers (PBDEs): Predicting partitioning in the environment. *Second Int. Workshop Brominated Flame Retardants* **2001**, 55−58.

(24) Meerts, I. A.; van Zanden, J. J.; Luijks, E. A.; Leeuwen-Bol, I.; Marsh, G.; Jakobsson, E.; Bergman, A.; Brouwer, A. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. *Toxicol. Sci.* **2000**, *56* (1), 95−104.

Received for review March 29, 2006. Revised manuscript received June 25, 2006. Accepted June 28, 2006. Financial support was given by the European commission RD (Life Science Program, QLK4-CT-2000-0261).

JF0608827