NON-ABSORBABLE FAT INCREASES THE DISPOSAL OF 2,2',4,4'-TETRABROMODIPHENYL (BDE-47) IN RATS THROUGH INTERRUPTION OF THE ENTEROHEPATIC CIRCULATION.

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Introduction

Organohalogens, like polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polybrominated diphenyl ethers (PBDEs), are structure related, stable, organic compounds. PCBs were first discovered in pike by Jensen in 1966[1]. Ever since many papers have been published on their existence and half-life in environment, animals and humans and on their potential hazard for animal and human health[2]. Based on demonstrated and suspected toxicity the production of PCBs was arrested in the 1970s. However, they are still ubiquitously present in the environment due to their slow decay[2].
Brominated flame retardants, for instance PBDEs, have been reported to share most of the qualities ascribed to PCBs. Nevertheless, their production has been intensified over the last two decades, due to their potency to diminish fire-related morbidity and mortality by 10% in a 1-year period, and their subsequent obligated use by the European Union. Since PBDEs are the most generally used flame retardants, their levels in environment are increasing, leading to higher exposure of animals and humans, and possibly to more health effects.

Theoretically the (potential) influence on human health could be alleviated by enhanced disposal of these lipophilic compounds from the body. This could be achieved by stimulating fecal fat excretion, which augments the lipophilic phase of the feces and consequently might extract lipophilic compounds from the body. We recently obtained experimental support for this concept by reporting on a novel, successful approach to enhance the disposal of an endogenous lipophilic compound, unconjugated bilirubin, from the body. In a rat model for unconjugated hyperbilirubinemia, the dietary administration of the lipase inhibitor orlistat induced a dose-dependent increase in fecal fat excretion, and, simultaneously, a dose-dependent decrease in plasma concentration of unconjugated bilirubin[3]. We hypothesized that the lipophilic unconjugated bilirubin is captured in unabsorbed fat in the intestinal lumen and subsequently excreted via the feces.
An alternative approach to increase fecal fat excretion could be by ingestion of non-absorbable fats, such as sucrose polyesters. Sucrose polyesters cannot be digested by physiological lipases in the intestine through spheric hindrance, and as a consequence cannot be absorbed by the intestine, leading to an augmented lipophilic phase of the feces. Two studies have reported on the use of the sucrose polyester olestra to enhance fecal excretion of organohalogens. In one study two patients with very high levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were treated with olestra in five different dosages, their daily TCDD excretion increased 8-10 fold[4]. In another study olestra was administered to three volunteers, increasing excretion of PCDD/Fs, PCBs and

hexachlorobenzene (HCB) 1.5 to 11-fold, depending on the compound[5]. Although these experiments support the feasibility of the concept, insights in the actual mechanism(s) of enhanced disposal were not obtained.

The mechanism(s) of enhanced disposal of a lipophilic compound could be related to an increase in its biliary secretion and/or its net transport over the intestinal wall. In the present study we aimed to determine the validity of this hypothesis. As model compound we choose ^{14}C-BDE-47, based on its increasing level in the environment and its long half-life in the human body. Fecal fat excretion was stimulated by either of two means, namely inhibition of digestion and subsequent absorption of dietary fat by orlistat, or administration of a non-absorbable sucrose polyester.

Methods and Materials

Animals. 13 male Wistar rats (body weight 320-405 g), were obtained from Harlan, The Netherlands. All animals were individually housed in an environmentally controlled facility with a 12-12 hours light/dark cycle (6.00 AM on, 6.00 PM off), with *ad libitum* access to food and water. The experimental protocol was approved by the Ethical Committee for Animal Experiments, Faculty of Medical Sciences, University of Groningen, The Netherlands.

Chemicals. Orlistat was obtained from Roche Nederland BV (Mijdrecht, The Netherlands). Liquid sucrose polyester, containing predominantly unsaturated long chain fatty acids, was a generous gift of Dr. J. Westrate, Unilever Research Laboratories (Vlaardingen, The Netherlands). All other chemicals were of analytical grade.

Diets. All diets were produced by Hope Farms BV (Woerden, The Netherlands). The control diet, code 4141.07, was a high-fat (12 wt% fat, 35 energy% fat), semisynthetic, purified diet containing 16% long-chain fatty-acids. The experimental lipase-inhibitor diet, code 4141.13, was identical to the control diet, except for the supplementation with 200 mg Orlistat (Xenical®)/kg. The experimental sucrose polyester diet, code 4141.07 SPE 4% unsaturated fat, was identical to the control diet, except for replacement of one third of the dietary fat (i.e. 4 wt%) by sucrose polyester.

Preparation of radiolabelled 14C-BDE-47. The radioactive labeled compound was developed at the department of Environmental Chemistry by Å. Bergman and his co-workers as part of a selection of organohalogens used in the European Union Compare project[6].

Experiment schedule. The animals received a high-fat diet during a 3 weeks run-in period, to best mimic human fat consumption. At day –4 15 μCi/kg bodyweight ^{14}C-BDE-47 via gavage was given, and the animals were randomly divided into three feeding groups at day 0 (group I: high-fat diet, group II: orlistat diet, group III: sucrose polyester diet). Feces was collected every weekday and heparin plasma every first day of the week by tail vein bleeding under isoflurane anesthesia. At the end of the experiment (day 22 for group I, day 23 for group II, day 24 for group III) the animals were bile-canulated under intraperitoneal pentobarbital anesthesia according to the method by Kuipers et.al.[7], and bile was collected for 30 minutes. Subsequently the animals were sacrificed by collecting approximately 10 ml of heparin blood by vena cava inferior punction, and liver, testicular fat, testicles, adrenal gland, intestine, thyroid and brains were collected.
All gathered materials and tissues were stored in prewashed acetone tubes at –20 ºC.

Feces. Feces was freeze dried, grinded and homogenized per day before storage at –20 ºC.

Determination of radioactive concentrations. Feces and bile were decolorized with bleach, and collected tissues were solved in solueen-350. To all samples Ultima Gold XR (Packard BioScience) scintillation fluid was added till samples were solved, after which scintillation was measured during 10 minutes in a liquid scintillation counter (Tricarb 2500, Packard). At the days blood samples were obtained fecal samples were also analyzed after lipid extraction according to Bligh-Dyer[8], for measurement of radioactivity in the organic and neutral phase of the feces.

Statistical analysis. Statistical analyses were performed by Mann Whitney with two-tailed exact significance. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Intestinal absorption of ^{14}C-BDE-47 after gastric gavage is virtually complete (>98% of administered dose), and fecal ^{14}C-BDE-47 excretion stabilizes within four days after administration (data obtained in pilot experiment, not shown). In the present study, rats were randomly assigned to the three different diets (high-fat, high-fat with orlistat, or high-fat with sucrose polyesters) at four days after gavage of ^{14}C-BDE-47. Table 1 shows that the amounts of feces produced by rats in either diet groups were similar. The amount of ^{14}C excreted via the feces, however, appeared approximately 2.7 and 2.3 fold higher in the sucrose polyesters group than in the control or the orlistat group, respectively. Cumulative ^{14}C-BDE-47 excretion between day 2 and 22 was also significantly higher in the sucrose polyesters-fed rats compared with the other two groups.

	Control	Orlistat	Sucrose polyesters
Feces production (g/day)	2.8 (0.4)	2.5 (0.6)	3.5 (1.0)
Fecal ^{14}C-BDE-47 excretion (dps/day)	105 (23)	143 (57) [A]	268 (71) [B]
Cumulative fecal excretion (% of administered dose)	1.59 (0.31)	1.89 (0.22) [C]	4.30 (0.44) [D]

Table 1. Feces production and fecal ^{14}C-BDE-47 excretion in rats, subsequently fed a control diet, or a diet containing orlistat or sucrose polyesters. Given values are means (standard deviations).
[A] orlistat compared to control diet, p=0.413.
[B] sucrose polyester compared to control diet, p=0.016.
[C] orlistat compared to control diet, p=0.202.
[D] sucrose polyester compared to control diet, p=0.000.

The data obtained allowed to estimate to what extent excretion of ^{14}C-BDE-47 involves biliairy excretion of the compound and/or transport over the intestinal wall into the intestinal lumen. No statistical difference in the excretion of ^{14}C-BDE-47 in the bile between control diet and orlistat diet (p=0.624) or between control diet and sucrose polyesters diet (p=0.806) was found. The excretion of ^{14}C-BDE-47 in bile was significantly lower in animals on the sucrose polyesters diet compared to animals on the orlistat diet (p=0.021). A compound secreted in the bile can be reabsorbed by the intestine and transported to the liver, resulting in the enterohepatic circulation. Taking this into consideration, we can deduct the total amount of BDE-47 prese
nt in the bile from the total amount of BDE-47 present in the feces and transport over the intestinal wall is computed. In figure 1 the result of these deductions are given.
As can be seen in figure 1, in each diet group there is a net transport of BDE-47 over the intestinal wall into the body. This transport is approximately the same for rats on the control diet and rats on the orlistat diet, consisting of about 75% of the dose in the bile. In the rats on the sucrose polyesters diet the net transport into the body is 11% of the dose in the bile.

Conclusion.

Dietary administration of non-absorbable sucrose polyesters increases the disposal of 2,2',4,4'-tetrabromodiphenyl (BDE-47) in rats through interruption of the enterohepatic circulation. Interestingly, stimulation of fecal fat excretion through drug-induced inhibition of intestinal lipases did not enhance the disposal of ^{14}C-BDE-47. The mechanisms underlying the apparent difference between the modes of increasing fecal fat excretion need further exploration.

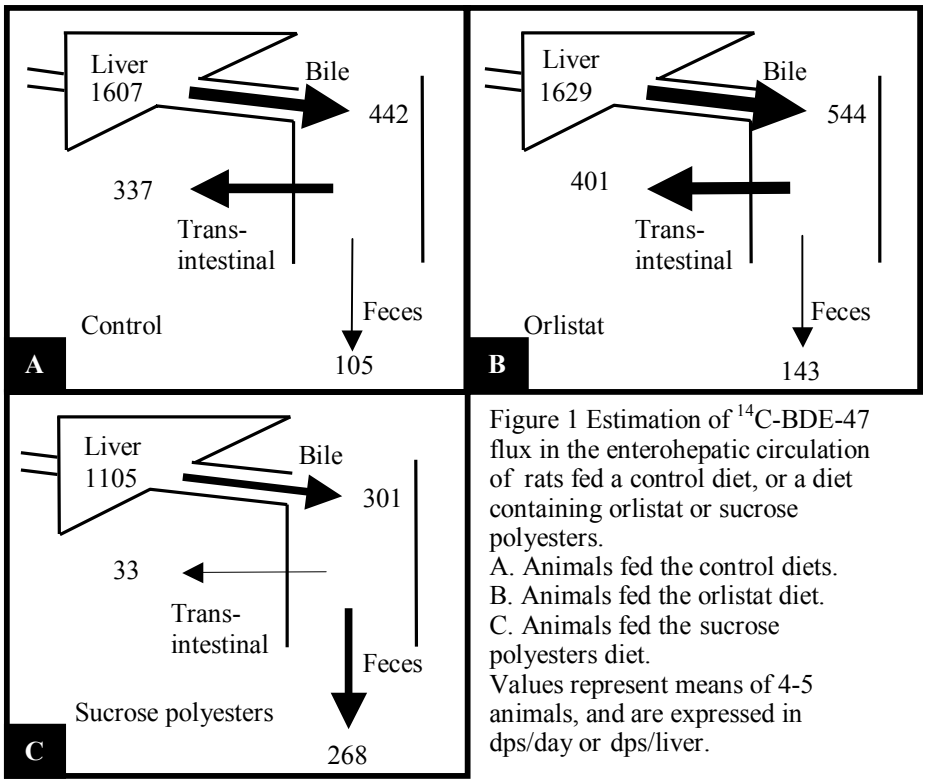

Figure 1 Estimation of ^{14}C-BDE-47 flux in the enterohepatic circulation of rats fed a control diet, or a diet containing orlistat or sucrose polyesters.
A. Animals fed the control diets.
B. Animals fed the orlistat diet.
C. Animals fed the sucrose polyesters diet.
Values represent means of 4-5 animals, and are expressed in dps/day or dps/liver.

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