Effects of Dose, Administration Route and/or Vehicle on Decabromodiphenyl Ether (DecaBDE) Concentrations in Plasma of Maternal, Fetal and Neonatal Rats and in Milk of Maternal Rats

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Running Title Page

Running title: Exposure Evaluation Study of DecaBDE in Rats

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Number of text pages: 23
Number of tables: 3
Number of figures: 4
Number of references: 28
Number of words in the Abstract: 209
Number of words in the Introduction: 632
Number of words in the Discussion: 1635

Non-standard abbreviations:
chronic oral reference dose (RfD)
corn oil (CO)
decabromodiphenyl ether (DecaBDE)
2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether congener (BDE-209)
developmental neurotoxicity (DNT)
European Union (EU)
gestation day (GD)
lactation day (LD)
limit of quantitation (LOQ)
Organisation for Economic Co-operation and Development (OECD)
postnatal day (PND)
soyaphospholipon:Lutrol® F127-water (SPL)
Supplemental Information (SI)
U.S. National Research Council (NRC)
U.S. National Toxicology Program (NTP)
Abstract

The effects of route and vehicle on blood and milk levels of decabromodiphenyl ether (DecaBDE; CASRN 1163-19-5) were investigated in the rat to assist in the design and conduct of a developmental neurotoxicity study. Blood plasma and/or milk concentrations were determined in the dams, fetuses, and/or nursing pups after repeated DecaBDE administration by gavage throughout gestation or gestation and lactation using corn oil (CO) or soyaphospholipon:Lutrol® F127-water (SPL) as the vehicle. The impact of vehicle on plasma levels was also investigated in pups derived from naïve dams after they received a single postnatal dose. This study reports for the first time that fetal and neonatal plasma concentrations are concurrent with those of maternal plasma and milk. Higher concentrations of DecaBDE were achieved in plasma and in milk with CO; than with SPL. Further, pups derived from dams treated with only SPL were lower in body weight, when compared to those from dams treated with either CO, CO and DecaBDE, or SPL and DecaBDE. This study demonstrates that oral administration of DecaBDE results in systemic exposure to gravid rats, fetuses and lactating pups. The study further exhibits that oral exposures of DecaBDE are relatively consistent for uptake across the dose range of 100 to 1000 mg/kg/day, when administered in CO.
Introduction

Decabromodiphenyl ether (DecaBDE; CASRN 1163-19-5) has been used as a flame retardant for approximately three decades. The primary component of the commercial DecaBDE product is the 2,2’,3,3’,4,4’,5,5’,6,6’-decabromodiphenyl ether congener (BDE-209). In 2000, the U.S. National Research Council (NRC) issued a chronic oral reference dose (RfD) for DecaBDE of 4 mg/kg/day (NRC, 2000). This value was derived from DecaBDE’s extensive toxicology database, which includes evaluations on subchronic and chronic toxicity, development, reproduction, mutagenicity, and carcinogenicity (Norris et al., 1973; Norris et al., 1974; Kociba et al., 1975; Norris et al., 1975; NTP, 1986; el Dareer et al., 1987; Hardy, 2002). The European Union (EU) independently reviewed these same studies and concluded that the manufacture and use of DecaBDE did not present a risk to human health (EC, 2002). The EU did, however, require manufacturers to conduct a guideline-compliant developmental neurotoxicity (DNT) study under Good Laboratory Practice standards (OECD, 1997; EC, 2006). In addition, the EU required evaluation of direct gavage administration to the pup and use of an atypical vehicle, soyaphospholipone-Lutrol® F 127 [SPL]. These requests were based on reports of preliminary developmental neurotoxicity findings in neonatal mouse pups directly administered BDE-209 (Viberg et al., 2001) and of BDE-209 absorption being enhanced when SPL was the vehicle (Mörck and Klasson-Wehler, 2001).¹

Until the report by Viberg et al. (2001), DecaBDE’s mammalian toxicology database supported its safe use as a flame retardant, as evidenced by NOELs and NOAELs of ≥1000 mg/kg/day in repeated dose studies (Hardy et al., 2009). DecaBDE’s limited bioavailability³ was considered a factor in its general lack of mammalian toxicity (NTP,
1986; el Dareer et al., 1987; Zhou et al., 2001). DecaBDE’s low toxicity, marginal uptake and low volume of distribution suggested that direct dosing of pups might be necessary to ensure adequate exposure.

The Organisation for Economic Co-operation and Development’s (OECD) guideline for developmental neurotoxicity testing requires maternal administration of the test substance from gestation day (GD) 6 through lactation day (LD) 21 (OECD, 2007). Adverse treatment-related pre- or postnatal outcomes are considered evidence of adequate exposure to the offspring, even when data on the dose received by the offspring are not available. Direct dosing of pre-weaning animals is sometimes used to resolve issues of the dose delivered to the offspring, but may complicate interpretation of results from a larger study (Zoetis and Walls, 2003).

A two-phase study was performed to characterize the most appropriate route (i.e., direct or indirect administration to pups) and vehicle (i.e., CO or SPL) for maximum delivery of DecaBDE to offspring. In phase one, plasma and/or milk concentrations were determined in the dams, fetuses, and/or nursing pups after repeated DecaBDE administration throughout gestation or during gestation and lactation using CO or SPL as the vehicle. Dose levels of 100, 300 and 1000 mg/kg/day in CO were used for this phase of the study based on the data generated from a previous prenatal developmental toxicity study in rats using similar doses (Hardy et al., 2002). In addition, a dose level of 1000 mg/kg/day in SPL served to provide comparative exposure data of DecaBDE when administered in CO versus SPL. In phase two, the impact of vehicle on plasma levels in pups derived from naïve dams was investigated after receiving a single oral (gavage) dose of DecaBDE on postnatal day (PND) 4. The objective of this work was to assist in the
design and performance of a DecaBDE DNT study in the rat by comparing the effects of time, dose and vehicle on plasma and milk levels, and to provide information on DecaBDE fetal and neonatal plasma levels concurrent with those in maternal plasma and milk for risk assessment use. In the process, a method for the analysis of microliter samples of plasma and milk was developed, and new insights into DecaBDE’s potential for toxicity were generated.
Materials and Methods

Chemicals

The test substance was a composite that contained equal proportions of the three commercial DecaBDE products (CASRN 1163-19-5; Figure S1) of Albemarle Corporation (Baton Rouge, LA), Chemtura Corporation (Middlebury, CT) and ICL-IP America, Inc. (St. Louis, MO). The composite was used to replicate previous studies (Hardy et al., 2002) and provide a test-substance representative of the three commercially available DecaBDE materials. The composite was determined to be 97.51% BDE-209 with 3 impurities (i.e., nonabromodiphenyl ethers) at approximately 2.5%. Prior to use, the composite was characterized for identity by Fourier transform infrared spectroscopy, and its purity and homogeneity were determined by gas chromatography. $^{13}$C$_{12}$DecaBDE ($>98\%$ BDE 209) was supplied by Cambridge Isotope Laboratories, Inc (Andover, MA). Corn oil was obtained from ACH Food Corporation (Memphis, TN). Phospholipon 90 NG and Lutrol$^\circledR$ F 127 NF Prill were obtained from American Lecithin Company (Oxford, CN) and Mutchler, Inc. (Harrington Park, New Jersey), respectively. Deionized water was prepared on site. Blank rat plasma and milk were acquired from Bioreclamation (East Meadow, NY).

Animals

Two hundred forty-five sexually mature, virgin female Crl:CD(SD) rats, approximately 70 day old at receipt, were obtained from Charles River Laboratories, Inc. (Raleigh, NC). Females, approximately 11 weeks of age, judged to be in good health and at a minimum of 220 grams were cohabitated (1:1) with in-house males. Resident males were
untreated, sexually mature rats used exclusively for mating. The presence of a vaginal copulatory plug or sperm in a vaginal lavage was considered positive evidence of mating. GD 0 was defined as the day on which evidence of mating was observed. After mating, females were randomly assigned to treatment groups based on stratification of the GD 0 body weights using a block design. Animals were housed in accordance with the Guide for the Care and Use of Laboratory Animals in animal facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (NRC, 1996). Animals had ad libitum access to Certified Rodent LabDiet® 5002 (PMI Nutrition International, LLC, Parkway Mulberry, FL) and reverse osmosis-purified drinking water. Animal room conditions were 71°F ± 5°F and 50% ± 20% relative humidity, 12-hour light/12-hour dark photoperiod, and at a minimum 10 fresh air changes/hour.

**Experimental Design**

Plasma concentrations after oral (gavage) administration to Sprague Dawley Crl:CD(SD) dams or pups using one of two vehicles, corn oil (CO) or soyaphospholipon Lutrol® F 127 (SPL), and single or multiple doses were investigated from 0 to 24 hr post-dosing. The doses administered to dams over GD 6 through LD 4, e.g., 0, 100, 300 or 1000 mg/kg/day in CO, were based on those of a prenatal developmental study with DecaBDE (Hardy et al., 2002).

The study consisted of eight groups of rats (Table 1). Four groups of bred female rats (n=14 in the control group and 48/DecaBDE group) were administered the test article in CO by oral gavage at dose levels of 0, 100, 300 or 1000 mg/kg/day. Half of the animals in each dose group were treated from GDs 6 through 20, while the remaining animals
continued on treatment through LD 4. On GD 20, half of the animals in each dose group were euthanized, and blood was collected from dams and fetal litters. The remaining animals were allowed to deliver, and dams and neonatal pups euthanized on LD 4. Blood was collected from dams and pups on LD/PND 4 and milk on PND 4 was also collected from dams. Two to three rats were sampled at each time point. Blood collection occurred immediately after dosing (0 hr), and 0.5, 1, 2, 4 or 8 hrs post-dosing. Milk collection occurred at 0.5, 1, 2, 4, 8 and 24 hrs post dosing. Two groups of bred female rats were administered the SPL vehicle with (n=24) or without (n=10) the test article at a dose of 1000 mg/kg/day over GD 6 through LD 4. Blood and milk collections were as described above for animals administered the test article in CO.

Two groups of eighteen naïve bred females were allowed to deliver, and their litters were randomly culled to 8 pups (4 males and 4 females, when possible)/litter on PND 4. On PND 4, pups were administered a single 20 mg/kg dose of the test article by gavage in either CO or SPL. The 20 mg/kg dose was selected based on Viberg et al. (2003) (Viberg et al., 2003). Blood was collected at sacrifice on PND 4 at 0.5, 1, 2, 4, 8 or 24 hrs post-dosing. Samples were analyzed for test article content, based on quantitation of BDE-209.

As an adjunct to the subsequent DNT study, plasma and/or milk concentrations at two additional doses, 1 and 10 mg/kg/day, administered GD 6 to LD 4, were determined in 3 or 4 F0 females/group and their litters 8 hrs post-dosing on LD 4.

Body weights and food consumption of the dams were measured on GD 0, 6, 9, 12, 15, 18 and 20, and on LD 1, 4, 7, 11, 14, 17, 21 and 22. Pups were individually weighed on PND 1, 4, 7, 11, 14 and 21.
**Blood and Milk Collection**

Maternal (lateral tail vein or vena cava) and fetal (umbilical vessels) blood samples were collected into chilled tubes containing lithium heparin. Pups were euthanized by decapitation, and trunk blood collected. Fetal and pup blood samples were pooled by litter. Plasma was isolated from whole blood in a refrigerated centrifuge. Females were separated from their litters 5.5 hours prior to milk collection, and oxytocin, 0.05 mL subcutaneously, was administered to stimulate milk let down. Milk was collected from the same dams as used for blood collection (4 dams/group/time point; vehicle control rats sampled at 4 hr post-dosing only). Milk was collected by separating the dams from their litters approximately 5.5 hours prior to milk collection. Immediately prior to collection, 1 U of oxytocin was administered to the dam by subcutaneous injection. The mammary glands were cleaned and then milk was collected using gentle massage over the glands until sufficient sample was achieved. Plasma and milk samples were stored frozen (-20 °C) until analyzed.

**Analysis of Test Article Formulations, Plasma, and Milk**

Test article-vehicle formulations were prepared weekly or as needed, divided into aliquots for daily use, and stored refrigerated and protected from light. Analyses were performed using gas chromatography with electron capture detection. The method was validated over the range of DecaBDE concentrations administered (Coffee, 2008). Rat plasma and milk samples were analyzed by gas chromatography (Agilent 6890N) - mass spectrometry (Agilent 5975C) equipped with an EI ionization source. The method was validated for 100 µL sample volumes prior to use over the concentration ranges of 1000
through 6000 ng DecaBDE/mL and 250 through 6000 ng DecaBDE/mL in rat plasma and milk, respectively (O’Lear, 2009). The method used liquid-liquid extraction of DecaBDE and $^{13}$C$_{12}$DecaBDE as the internal standard. Valid analytical runs were required to have at least two-thirds of the QC samples and at least one-third at each level to be within 85% to 115% of the target QC concentration.

Details on the Materials and Method as well as additional endpoints evaluated in the study are found in the Supplemental Information (SI).
Results

Mortality, Morbidity, Body Weights, Food Consumption, Gestational and Litter Data

No test-substance related deaths or clinical signs of toxicity occurred in the F0 females, when CO was the vehicle used to deliver the test-substance. Also no test substance-related differences in mean body weight, body weight gain or food consumption were detected during gestation or lactation (Tables S1-S3). When SPL was the vehicle, mean food consumption by maternal animals was increased in the 1000 mg/kg/day group compared to the SPL control group on LDs 11 through 24, 14 through 17, 17 through 21, 21 through 22 and 1 through 22 (p<0.05 or 0.01) (Table S3). The trend in food consumption for the SPL 1000 mg/kg/day group was similar to that of the CO control over these intervals (Table S3), as was body weight gain (Tables S1-S2).

DecaBDE administration in CO or SPL did not affect gestation or litter parameters (Table S4). Mean gestational length in treated animals was comparable to their respective vehicle controls and similar to the laboratory’s historical control database (mean = 21.9 days). The mean number of pups born, percentage of males at birth, mean live litter size and postnatal survival were similar between control groups and their respective treatment groups.

Mean offspring body weights and weight gains through PND 21 in the CO treated groups were similar to CO controls and/or laboratory historical control means (Table 2; Table S5). Body weights of male and female pups in the SPL 1000 mg/kg/day group were higher than their respective SPL control group on PND 7, 11 (females only), 14 and 21 (p<0.05 or 0.01). Body weight gains were also increased in the SPL 1000 mg/kg/day male and female pups on PNDs 4 through 7 and 17 through 21. In contrast, mean body weights
in the SPL control group were consistently lower than that of litters derived from CO control dams. Furthermore, body weight gains by SPL control pups of both sexes were found to be approximately 66% to 88% of the CO control pups’ gains.

**Plasma Concentrations in Dams and Fetal Litters on GD20**

DecaBDE plasma concentrations in dams and their litters just prior to and up to 8 hrs post-dosing on GD 20 with CO as the vehicle are shown in Figure 1 (Table S6). The dams’ mean plasma concentrations were similar at each time point regardless of dose (100, 300 or 1000 mg/kg/day). A similar lack of dose and time response was seen in fetal plasma, but levels were typically 2.5 to 5-fold lower than the dams’. Further, concentrations of DecaBDE in both maternal and fetal plasma were relatively consistent across dose levels (Table 3).

**Plasma and Milk Concentrations in Dams on LD 4**

Plasma concentrations in dams at 0.5 to 24 hrs post-dosing on LD 4 after administration in CO over GD 6 through LD 4 are shown in Figure 2 (Tables S7-S8). The plasma concentration patterns of the dams administered the test article in CO were similar to those on GD 20. LD 4 plasma concentrations at each time point were similar at 100, 300, and 1000 mg/kg/day. This is demonstrated in Table 3 - that is, when a comparison was made of the AUC_{last} for the CO test-substance groups across a 10-fold dose range, exposures on LD 4 after multiple daily doses were relatively similar. In contrast, exposure to DecaBDE for dams administered 1000 mg/kg/day over GD 6 through LD 4 in SPL was 4- to 5-fold lower than in CO (Figure 3 and Table 3).
Mean milk concentrations at doses of 100 and 1000 mg/kg/day in CO were relatively stable between 0.5 and 8 hrs post-dosing, and exposure in milk was similar irrespective of dose (Figure 4 and Table 3). Milk concentrations were generally lower than plasma concentrations. In contrast, milk concentrations in females administered DecaBDE in SPL at 1000 mg/kg/day over GD 6 through LD 4 were below the limit of quantitation (LOQ) in 71% of the samples (Table S8). Where detected, the levels were 45 to 60% of those in the CO 1000 mg/kg/day group.

LD 4 blood and milk were collected at 8 hrs post-dosing in the DNT study from dams administered DecaBDE in CO at 1 or 10 mg/kg/day from GD 6 through LD 4 (Tables S7-S8). The mean plasma (1700 ± 540 ng/mL) and milk (1250 ± 195 ng/mL) concentrations in the CO 10 mg/kg/day group were similar to those at CO 100 to 1000 mg/kg/day groups. The mean plasma (510 ± 89 ng/mL) and milk (<LOQ – 509 ng/mL) concentrations at 1 mg/kg/day were lower.

Plasma Concentrations in Corresponding PND 4 Pups

The PND 4 mean plasma concentrations in the CO 100 and 1000 mg/kg/day groups were similar over dose groups and time, suggesting a lack of dose response and consistency among exposures across the dose range (Figure 2; Table 3; Table S7). Pup plasma concentrations and AUC last were roughly 2-fold higher than in dams’ plasma (Table 3). Like the CO 1000 mg/kg/day group, the mean PND 4 plasma concentrations in nursing pups from the SPL 1000 mg/kg/day group were roughly stable over time and higher than the dams’ plasma concentrations (Figure 3). However, the PND 4 pups’ mean plasma
concentrations in the SPL 1000 mg/kg/day group were clearly (approximately 3-fold) lower than the CO 1000 mg/kg/day group (Table S7).

PND 4 plasma samples were collected from pups at 8 hrs post-dosing of the maternal females with CO at doses of 1 or 10 mg/kg/day in the DNT study (Table S7). Like the dams’ LD 4 plasma and milk, the pup mean plasma concentration (2140 ± 257 ng/mL) in the CO 10 mg/kg/day group was similar to those of the CO 100 and 1000 mg/kg/day groups, whereas that in the 1 mg/kg/day group (929 ± 124 ng/mL) was approximately 2.5-fold lower (Table S7).

**Plasma Concentrations in Pups on PND 4 After a Single Dose**

Plasma concentrations following a single 20 mg/kg dose in CO to pups on PND 4 increased with time post-dosing from <LOQ to 1845 ± 323 ng/mL at 0.5 and 24 hrs post-dosing, respectively (Table S9). The peak level was within the range of PND 4 pups derived from dams administered the test substance in CO from GD 6 through LD 4. Plasma concentrations on PND 4 ranged from <LOQ to 293 ± 29 ng/mL after a single 20 mg/kg dose in SPL and were clearly lower than those achieved with CO. Both the AUC$_{0-8h}$ and the AUC$_{0-24h}$ were lower in directly dosed pups, compared to pups receiving DecaBDE indirectly via the dams (Table 3). Additional details on these results, including tabulated values for figures and data not shown, are provided in the SI.
Discussion

Several studies have reported limited absorption of DecaBDE after oral administration (NTP, 1986; el Dareer et al., 1987; Huwe et al., 2008). For example, the U.S. National Toxicology Program (NTP) reported dietary absorption of approximately 0.33% of the dose by rats. However, 10% absorption from a single gavage dose, when using SPL as the delivery vehicle, was reported in rats based on levels detected in bile (Mörck et al., 2003). SPL was chosen in that study after demonstrating higher solubility of DecaBDE in SPL, when compared to two other vehicles. The 10% value was subsequently interpreted as representing systemic absorption (i.e., bioavailability), but did not take into account the negligible blood levels reported by Mörck et al. (2003) and others during this interpretation. Also not taken into consideration was the likelihood of extensive first-pass elimination, as suggested by the concentrations in bile versus blood. But based on these data, the EU was still interested in SPL’s use as the vehicle in a guideline-compliant DNT study to assure maximum exposure to pups.

The present study was conducted to determine the most appropriate vehicle (CO or SPL) and method of administration (maternal or pup) for the DNT study by investigating blood plasma and/or milk levels in the dam and/or pups. Information on gestational and lactational parameters as a function of dose and vehicle was also obtained. These data demonstrated that administration of DecaBDE in CO at doses of 100, 300 and 1000 mg/kg/day from GDs 6 through 20 or GD 6 through LD 4 did not adversely affect the maternal animals or their litters. The absence of maternal or fetal toxicity was consistent with the results of an earlier prenatal developmental study where doses up to 1000 mg/kg/day were administered from GDs 0 through 20 (Hardy et al., 2002).
While not compared statistically, visual inspection of the data showed that mean body weights in lactating SPL control dams were typically lower than CO administered dams. Food consumption in the SPL control dams was also lower than CO control or CO 1000 mg/kg/day dams, while food consumption in the SPL 1000 mg/kg/day dams approached that of the dams treated with test-substance using CO. Further, mean body weights of pups derived from dams administered repeated doses of the SPL vehicle were lower than pups derived from CO test-substance treated dams over PNDs 7 through 21. This reduction in body weight was not observed in pups derived from dams administered DecaBDE in the CO vehicle.

The SPL-induced reduction in pup body weight precluded its use in the DNT study, and on this basis alone CO appeared a more suitable vehicle. However, SPL also negatively affected plasma and milk concentrations in the dams and/or pups. DecaBDE plasma concentrations in LD 4 dams and PND 4 pups were lower when animals were administered 1000 mg/kg/day over GD 6 through LD 4 using SPL as the vehicle, instead of CO. DecaBDE concentrations in milk were also lower than those achieved with CO, when compared to DecaBDE concentrations administered to dams in SPL over GD 6 through LD 4. Similarly, DecaBDE plasma concentrations were lower in naïve pups administered a single direct dose on PND 4, when SPL was the vehicle instead of CO. These plasma concentrations were also substantially lower than those of pups derived from dams administered DecaBDE in CO at 1000 mg/kg/day from GD 6 through LD 4. Thus, contrary to the expected increase in bioavailability, SPL produced lower DecaBDE concentrations, often below detection, in maternal and neonatal plasma and/or milk than equivalent doses administered in CO.
Consistent DecaBDE plasma levels over a 24 hr period were demonstrated during gestation and early into lactation for the first time in the rat. In addition, exposure to DecaBDE, as demonstrated in maternal, fetal and neonatal plasma and in maternal milk samples was similar across a 10-fold dose range when administered in CO. Because concentrations in these compartments did not fluctuate significantly on the days evaluated, the profiles were suggestive of having reached steady state. Attainment of steady-state levels during this time frame is consistent with elimination of >99% of a dietary dose within 72 hrs (NTP 1986), as well as Huwe et al.’s (2008) report that notes brominated congeners BDE-28/33, -47, -99, -100, -138, -153, -154, -183 and -197 reached steady-state in rat adipose tissue by day 14 of treatment. Given that steady-state is typically reached within 5 to 7 half-lives, Huwe et al.’s (2008) results further suggest that adipose half-lives of 0.4 to 2.8 days are likely for these 10 lower brominated congeners. Although BDE-209 was included in the test mixture administered in the Huwe et al. (2008) study, it was not included in the authors’ estimate of time-to-steady-state or adipose half-life because it was not detected in adipose tissue. These half-lives represent a worst-case, because adipose tissue is poorly perfused with typically longer (re)distribution time than other tissues. When viewed in total, our data along with other reports (Norris et al., 1973; Norris et al., 1974; Norris et al., 1975; el Dareer et al., 1987) demonstrate DecaBDE, and presumed lower brominated diphenyl ether congeners, do not have half-lives consistent with highly bioaccumulative substances, and suggest that a concern for unrealized adverse effects due to extremely long half-lives is not relevant.

Also for the first time, DecaBDE plasma concentrations were shown to plateau at oral doses ≥10 mg/kg/day. Plasma levels in rats were generally indistinguishable over a
dose range of two orders of magnitude, i.e., 10 to 1000 mg/kg/day, in dams, fetal litters and neonatal pups. Fetal plasma and maternal milk concentrations were lower than maternal plasma concentrations, whereas neonatal plasma concentrations were similar to, or higher than, maternal plasma concentrations. The lack of dose response in maternal plasma concentrations may be due to a combination of factors, including binding to fecal macromolecules, diffusion-limited uptake from the gut into the portal circulation, and efficient first-pass elimination in the bile, which results in only a small fraction of the dose being available systemically.

DecaBDE is expected to bind to particulates, including those found in fecal matter, and such evidence for this is seen in studies reporting a substantial fraction of the dose that was not able to be extracted from the feces (NTP, 1986; el Dareer et al., 1987; Mörck et al., 2003; Huwe and Smith, 2007; Huwe et al., 2008). DecaBDE’s binding properties have, however, led to different conclusions regarding its fate in the body. For example, Huwe and Smith (2007) reported non-extraction of BDE-209 from feces as evidence for metabolism; this conclusion was subsequently reinterpreted later as poor recovery by the same authors (Huwe et al., 2008). Similarly, Mörck et al. (2003) concluded that 65% of an oral dose of 14C-BDE-209 was present in the gut as metabolites. The author defined metabolites as all 14C-activity not extracted with the parent molecule, including that engaged in nonspecific binding. However, other studies using 14C-labelleled test-article have demonstrated that after oral administration, DecaBDE is poorly metabolized and predominantly excreted in the feces as parent molecule (NTP, 1986; el Dareer et al., 1987; Huwe et al., 2008; Riu et al., 2008).
Bioavailability after oral dosing is dependent on the rate and extent of absorption and systemic clearance (van de Waterbeemd and Testa, 2009). Absorption is dependent on solubility, permeability, gut wall metabolism, and cellular transporters. The rate of absorption from the gut lumen can be limited by the dissolution rate. For poorly soluble compounds such as DecaBDE, dissolution can be the rate-limiting factor for absorption. Absorption is also dependent on the permeability of the substance through the intestinal tract membrane. Molecular factors affecting permeability are solubility, flexibility, H bonding, molecular size/shape, and lipophilicity. DecaBDE’s molecular weight (959.2) likely imparts a negative effect on its permeability, as does the molecule’s non-coplanar spatial arrangement and limited solubility (water solubility <0.1 μg/L). These properties suggest DecaBDE’s oral absorption would be slow and limited, as was found in the present study and previously reported by others (NTP, 1986). Further, the present results suggest DecaBDE’s absorption may be governed by zero-order kinetics. That is, at doses ≥10 mg/kg/day, a constant amount of DecaBDE was absorbed per unit time, whereas first order kinetics (a constant fraction of the administered dose) likely governed absorption at the dose of 1 mg/kg/day. Sandholm et al. (2003)’s Figure 1 suggests first order absorption kinetics at a dose of approximately 2 mg/kg based on the similar terminal slopes of the oral and IV concentration-time curves (Sandholm et al., 2003). If zero-order kinetics were operative at this dose, different slopes would be expected. Therefore, the transition point between first- and zero-order absorption kinetics appears to occur between 1 (or possibly 2) and 10 mg/kg/day.

A similar lack of dose response in fetal plasma and maternal milk suggest maternal plasma concentrations dictate DecaBDE concentrations in these compartments. The
generally lower concentrations detected in fetal plasma and milk also suggests that DecaBDE’s distribution from the maternal circulation into these compartments is limited. These results are consistent with Riu et al. (2008), who reported 0.43% distribution of the dose to fetal litters. The higher plasma concentrations of DecaBDE in neonates (nursing pups) compared to the plasma concentrations in dams suggests, as expected due to immature hepatic excretory function, that these pups eliminate DecaBDE more slowly than adult rats.

In conclusion, new information was generated on the relationships between maternal, fetal and neonatal plasma and maternal milk DecaBDE concentrations after repeated dosing in the rat. This study suggests that DecaBDE steady-state plasma concentrations in the rat are achieved within 14 daily doses, and possibly sooner. Also for the first time, maximal DecaBDE plasma concentrations were shown to occur in rat plasma and milk at oral doses as low as 10 mg/kg/day. Increasing the oral dose to 1000 mg/kg/day did not result in a corresponding increase in plasma or milk DecaBDE concentrations.
Acknowledgements

JAB, JMA, HS, SJ, MH, and TS are employed by specialty chemical manufacturers whose product lines include brominated flame retardants. MJB, DGS, NRM, ESB, and DWS are employed by WIL Research Laboratories, a contract research organization commissioned to conduct the study presented herein. The views and opinions expressed in this article are those of the authors and not necessarily those of their respective employers.
DMD #33431

References


O'Lear JR (2009) Development and validation of GC/MS assays for the determination of decabromodiphenyl oxide (DEBDPO) concentration in rat plasma and rat milk (Study No. WIL-635003), WIL Research Laboratories, LLC, Ashland, OH.

OECD (1997) Principles on Good Laboratory Practice. *OECD(C(97)186/Final)*.

Disposition and metabolic profiling of [14C]-decabromodiphenyl ether in pregnant 

Sandholm A, Emanuelsson BM and Wehler EK (2003) Bioavailability and half-life of 

van de Waterbeemd H and Testa B (2009) Chapter 1 Introduction: The why and how of 
drug bioavailability research, in: *Drug Bioavailability - Estimation of solubility, 
permeability, absorption and bioavailability, Second, Completely revised edition* 
(van de Waterbeemd H and Testa B eds), pp 1-6, Wiley-VCH Verlag GmbH & Co., 
Weinheim, Germany.

retardant uptake, retention and developmental neurotoxic effects of 
decabromodiphenyl ether (PBDE 209) in the neonatal mouse., in: *Second 
International Workshop on Brominated Flame Retardants*, pp 279-281, Stockholm 
University, Sweden.

derangements in adult mice receiving decabrominated diphenyl ether (PBDE 209) 
during a defined period of neonatal brain development. *Toxicol. Sci.* **76**:112-120.

exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic 
Footnotes

a) Unnumbered footnote.

The Bromine Science and Environmental Forum funded the research described herein.

b) Unnumbered footnote (Meeting abstracts where the work was previously presented).


c) The name and full address and e-mail address of person to receive reprint requests.

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d) Numbered footnotes.

1Reference Dose (RfD): “An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used.” Source: http://www.epa.gov/IRIS/help_gloss.htm#r (Accessed March 2, 2010).

2Published as Viberg et al. (2003) and Mörck et al. (2003), respectively.
Throughout this report, the classical pharmacokinetic definitions for bioavailability and oral absorption will be used. Bioavailability is defined as the fraction and rate of the administered dose reaching the systemic circulation as the parent molecule. Oral absorption is defined as that fraction of the dose reaching the portal vein as the parent molecule.
Legends for Figures

Figure 1. DecaBDE plasma concentrations (ng/mL) on GD 20 in F₀ dams (D) and fetuses (F) after administration of DecaBDE in CO at 100, 300 and 1000 mg/kg/day to F₀ dams from GDs 6 through 20. Mean ± SD.

Figure 2. DecaBDE plasma concentrations (ng/mL) on LD 4 in F₀ dams (D) and pups (P) after administration of DecaBDE in CO at 100, 300 and 1000 mg/kg/day to F₀ dams from GD 6 through LD 4. Mean ± SD.

Figure 3. Comparison of DecaBDE plasma concentrations in dams (D) and pups (P) on LD 4/PND 4 following administration of DecaBDE in CO or SPL at 1000 mg/kg/day to F₀ dams over GD 6 through LD 4. Mean ± SD.

Figure 4. Comparison of DecaBDE plasma and milk concentrations (ng/mL) on LD 4 of F₀ dams after administration in CO at 100, 300 and 1000 mg/kg/day or SPL at 1000 mg/kg/day over GD 6 through LD 4. Mean ± SD.
## TABLE 1. Description of the DecaBDE dosage regimens, sample types collected and sampling intervals in F₀ females, fetuses and/or neonates.

<table>
<thead>
<tr>
<th>Group number</th>
<th>Dosage level (mg/kg/day)</th>
<th>Vehicle</th>
<th>F₀ females; Multiple doses</th>
<th>Naïve littersᵃ (8 Pups/Litter); Single direct dose to pups on PND 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GD 6 through 20*</td>
<td>GD 6 through LD 4**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. Females</td>
<td>GD 20 Samplesᵇ</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>CO</td>
<td>4ᵈ</td>
<td>Blood</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>CO</td>
<td>24</td>
<td>Blood</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>CO</td>
<td>24</td>
<td>Blood</td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>CO</td>
<td>24</td>
<td>Blood</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>CO</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>SPL</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>SPL</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>8</td>
<td>1000</td>
<td>SPL</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>A₁ᵇ</td>
<td>1</td>
<td>CO</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>A₂ᵇ</td>
<td>10</td>
<td>CO</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

ᵃSamples were collected at 0 (pre-dose) and at 0.5, 1, 2, 4 and 8 hours post dosing on each day of sampling, except as indicated for controls.

ᵇSamples were collected at 0.5, 1, 2, 4, 8 and 24 hours post dosing on each day of sampling, except as indicated for controls.

ᶜNot previously exposed to DecaBDE.

ᵈF₀ females and fetuses (GD 20).

ᵉF₀ females (LD 4); pups (PND 4).

ᶠBlood collected only at 1 hour.

ᵍNot performed.

恓=A=adjunct; samples collected at 8 hours post dosing only on LD 4/PND 4.

CO = Corn oil

SPL = Soyaphospholipon:Lutrol® F127 (16:34 w/w)/mL water
TABLE 2. F1 pup body weights (g), postnatal days (PNDs) 1 through 21. F1 pups derived from females administered DecaBDE at doses of 100 to 1000 mg/kg/day in corn oil (CO) or 1000 mg/kg/day in soyaphospholipon:Lutrol® F127 (16:34 w/w) water (SPL) from GD 6 through LD 22.

<table>
<thead>
<tr>
<th>PND</th>
<th>Maternal Dose (mg/kg/day, GD 6 through LD 21) and Vehicle</th>
<th>F1 Males&lt;sup&gt;b&lt;/sup&gt;</th>
<th>F1 Females&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO (n=9)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CO 100 (n=22)</td>
<td>CO 300 (n=24)</td>
</tr>
<tr>
<td></td>
<td>7.6±0.27</td>
<td>7.5±0.14</td>
<td>7.3±0.10</td>
</tr>
<tr>
<td></td>
<td>10.3±0.48</td>
<td>10.4±0.23</td>
<td>9.9±0.19</td>
</tr>
<tr>
<td></td>
<td>16.9±0.90</td>
<td>16.9±0.34</td>
<td>16.2±0.32</td>
</tr>
<tr>
<td></td>
<td>26.6±1.27</td>
<td>26.6±0.61</td>
<td>25.7±0.45</td>
</tr>
<tr>
<td></td>
<td>33.6±1.51</td>
<td>33.4±0.79</td>
<td>31.9±0.54</td>
</tr>
<tr>
<td></td>
<td>41.1±1.79</td>
<td>39.4±0.93</td>
<td>38.1±0.66</td>
</tr>
<tr>
<td></td>
<td>51.6±2.25</td>
<td>50.7±1.19</td>
<td>48.8±0.74</td>
</tr>
<tr>
<td>7</td>
<td>16.0±0.86</td>
<td>15.8±0.27</td>
<td>15.3±0.32</td>
</tr>
<tr>
<td>11</td>
<td>25.6±1.39</td>
<td>25.1±0.56</td>
<td>24.4±0.53</td>
</tr>
<tr>
<td>14</td>
<td>32.1±1.87</td>
<td>32.2±0.67</td>
<td>30.3±0.61</td>
</tr>
<tr>
<td>17</td>
<td>39.0±1.99</td>
<td>38.3±0.79</td>
<td>36.4±0.75</td>
</tr>
<tr>
<td>21</td>
<td>49.2±2.51</td>
<td>49.2±1.03</td>
<td>46.6±0.95</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of litters.
<sup>b</sup>Mean ± S.E.M.

*Significantly different from SPL control (p<0.05).
Table 3. Summary of toxicokinetic parameters of DecaBDE for plasma and milk from dams on GD 20 or LD 4 and plasma from pups on GD 20 or PND 4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Maternal plasma, GD 20</th>
<th>Fetal plasma, GD 20</th>
<th>Maternal plasma, LD 4</th>
<th>Maternal milk, LD 4</th>
<th>Pup plasma, PND 4</th>
<th>Pup plasma, PND 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group</td>
<td>Group</td>
<td>Group</td>
<td>Group</td>
<td>Group</td>
<td>Group</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;ave&lt;/sub&gt; hr*ng/mL</td>
<td>15393</td>
<td>13299</td>
<td>11974</td>
<td>4087</td>
<td>3371</td>
<td>2934</td>
<td>30559</td>
</tr>
<tr>
<td>C&lt;sub&gt;ave&lt;/sub&gt; ng/mL</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>432</td>
<td>315</td>
<td>---</td>
<td>1273</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; ng/mL</td>
<td>2299</td>
<td>1885</td>
<td>1666</td>
<td>839</td>
<td>649</td>
<td>426</td>
<td>1812</td>
</tr>
<tr>
<td>C&lt;sub&gt;min&lt;/sub&gt; ng/mL</td>
<td>832</td>
<td>1147</td>
<td>949</td>
<td>436</td>
<td>343</td>
<td>260</td>
<td>715</td>
</tr>
<tr>
<td>Fluctuation %</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>93.2</td>
<td>39.9</td>
<td>---</td>
<td>86.2</td>
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<tr>
<td>Dose norm AUC&lt;sub&gt;ave&lt;/sub&gt;</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>154</td>
<td>44.3</td>
<td>12.0</td>
<td>40.9</td>
<td>11.2</td>
<td>2.93</td>
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<tr>
<td>Dose norm C&lt;sub&gt;ave&lt;/sub&gt;</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>4.32</td>
<td>1.05</td>
<td>---</td>
<td>12.7</td>
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<tr>
<td>AUC&lt;sub&gt;0-8hrs&lt;/sub&gt; hr*ng/mL</td>
<td>34823</td>
<td>34823</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Dose norm AUC&lt;sub&gt;0-8hrs&lt;/sub&gt;</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24hrs&lt;/sub&gt; hr*ng/mL</td>
<td>8444</td>
<td>466</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
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</tr>
<tr>
<td>Dose norm AUC&lt;sub&gt;0-24hrs&lt;/sub&gt;</td>
<td>34823</td>
<td>3451</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;max&lt;/sub&gt;</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
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<td>---</td>
</tr>
<tr>
<td>Dose norm C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>---</td>
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<td>---</td>
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<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

<sup>a</sup>Not calculated  
<sup>b</sup>Not applicable
Figure 1.
Figure 2.

The graph shows the concentration of DecaBDE in plasma (ng/mL) over time (hours post-dosing). Different doses are indicated by different symbols and line styles.

- **D-100** (solid green line with green circles)
- **D-300** (dashed blue line with blue squares)
- **D-1000** (dotted red line with red triangles)
- **P-100** (dashed green line with green circles)
- **P-300** (dotted blue line with blue squares)
- **P-1000** (dashed red line with red triangles)

The x-axis represents hours post-dosing, ranging from 0 to 20, and the y-axis represents the concentration of DecaBDE in plasma, ranging from 100 to 10,000 ng/mL.
Figure 3.
Figure 4.