10-2010

# 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

J. A. Biesemeier

Melissa J. Beck
*Cedarville University*, mbeck@cedarville.edu

H. Silberberg

N. R. Myers

J. M. Ariano

***See next page for additional authors***

**Authors**
J. A. Biesemeier, Melissa J. Beck, H. Silberberg, N. R. Myers, J. M. Ariano, E. S. Bodle, D. W. Sved, S. Jacobi, D. G. Stump, M. Hardy, and T. Stedeford

0090-9556/10/3810-1648–1654$20.00
DRUG METABOLISM AND DISPOSITION
Vol. 38, No. 10
33431/3620866
*Printed in U.S.A.*

DMD 38:1648–1654, 2010



# Effects of Dose, Administration Route, and/or Vehicle on Decabromodiphenyl Ether Concentrations in Plasma of Maternal, Fetal, and Neonatal Rats and in Milk of Maternal Rats[S]

John A. Biesemeier, Melissa J. Beck, Hanna Silberberg, Nicole R. Myers, John M. Ariano, Eric S. Bodle, Daniel W. Sved, Sylvia Jacobi, Donald G. Stump, Marcia Hardy, and Todd Stedeford

*Chemtura Corporation, West Lafayette, Indiana (J.A.B., J.M.A.); WIL Research Laboratories, LLC, Ashland, Ohio (M.J.B., N.R.M., E.S.B., D.W.S., D.G.S.); ICL-IP America, Inc., Potomac, Maryland (H.S.); Albemarle Europe SPRL, Louvain-la-Neuve Sud, Belgium (S.J.); and Albemarle Corporation, Baton Rouge, Louisiana (M.H., T.S.)*

Received March 18, 2010; accepted June 24, 2010

**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 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 F 127-water (SPL) as the vehicle. The impact of vehicle on plasma levels was also investigated in pups derived from naive dams after a single postnatal dose. This study reports for the first time fetal and neonatal plasma concentrations concurrent with those of maternal plasma and/or milk. Higher concentrations of DecaBDE were achieved in plasma and in milk with CO than with SPL. Furthermore, pups derived from dams treated with only SPL were lower in body weight, compared with those from dams treated with either CO, CO and DecaBDE, or SPL and DecaBDE. The study further shows that exposure to DecaBDE is relatively consistent 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 issued a chronic oral reference dose[1] for DecaBDE of 4 mg/(kg · day) (National Research Council, 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, 1974, 1975; Kociba et al., 1975; National Toxicology Program, 1986; el Dareer et al., 1987; Hardy et al., 2002, 2009). 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 (European Commission, 2002). However, the EU did re-

This work was funded by The Bromine Science and Environmental Forum.

Parts of this work were previously presented at the following conference: Beck MJ, Silberberg H, Biesemeier J, Jacobi S, and Stedeford S (2009) An oral developmental neurotoxicity study of decabromodiphenyl ether (DecaBDE) in rats, including assessment of maternal and neonatal exposure profiles. *39th Annual Meeting*; 2009 Oct 17–21; Chicago, IL. Society for Neuroscience, Washington, DC.

J.A.B., J.M.A., H.S., S.J., M.H., and T.S. are employed by specialty chemical manufacturers whose product lines include brominated flame retardants.

M.J.B., N.R.M., E.S.B., D.W.S., and D.G.S. 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.

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.

doi:10.1124/dmd.110.033431.

[S] The online version of this article (available at http://dmd.aspetjournals.org) contains supplemental material.

[1] Reference dose: "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 no-observed-adverse-effect, lowest-observed-adverse-effect, 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.

**ABBREVIATIONS:** DecaBDE, decabromodiphenyl ether; BDE-209, 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether congener; EU, European Union; DNT, developmental neurotoxicity; SPL, soyaphospholipon/Lutrol F 127-water; GD, gestation day; LD, lactation day; CO, corn oil; PND, postnatal day; LOQ, limit of quantitation; BDE-28, 2,3,5-tribromodiphenyl ether; BDE-33, 2,3′,4′-tribromodiphenyl ether; BDE-47, 2,2′,4,4′-tetrabromodiphenyl ether; BDE-99, 2,2′,4,4′,5-pentabromodiphenyl ether; BDE-100, 2,2′4,4′,6-pentabromodiphenyl ether; BDE-138, 2,2′,3,4,4′,5-hexabromodiphenyl ether; BDE-153, 2,2′,4,4′,5,5′-hexabromodiphenyl ether; BDE-154, 2,2′,4,4′,5,6′-hexabromodiphenyl ether; DE-183, 2,2′,3′,4,4′,5′,6-heptabromodiphenyl ether; BDE-197, 2,2′,3,3′,4,4′6,6′-octabromodiphenyl ether.

quire manufacturers to conduct a guideline-compliant developmental neurotoxicity (DNT) study under Good Laboratory Practice standards (Organisation for Economic Co-operation and Development, 1997; European Commission, 2006). In addition, the EU required evaluation of direct gavage administration to the pup and use of an atypical vehicle, soyaphospholipon/Lutrol F 127-water (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).[2]

Until the report by Viberg et al. (2001), DecaBDE's mammalian toxicology database supported its safe use as a flame retardant, as evidenced by no-observed-effect levels and no-observed-adverse-effect levels of ≥1000 mg/(kg · day) in repeated-dose studies (Hardy et al., 2009). DecaBDE's limited bioavailability[3] was considered a factor in its general lack of mammalian toxicity (National Toxicology Program, 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 guideline for developmental neurotoxicity testing requires maternal administration of the test substance from gestation day (GD) 6 through lactation day (LD) 21 (Organisation for Economic Co-operation and Development, 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 preweaning 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., corn oil (CO) or SPL] for maximum delivery of DecaBDE to offspring. In phase 1, 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 2, the impact of vehicle on plasma levels in pups derived from naive dams was investigated after a single oral (gavage) dose of DecaBDE in CO or SPL 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.

[2] Published as Viberg et al. (2003) and Mörck et al. (2003), respectively.

[3] Throughout this report, the classic 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.

## Materials and Methods

**Chemicals.** The test substance was a composite that contained equal proportions of the three commercial DecaBDE products (CASRN 1163-19-5) (Supplemental Fig. 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 three impurities (i.e., nonabromodiphenyl ethers) at approximately 2.5%. Before 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 F 127 NF Prill were obtained from American Lecithin Company (Oxford, CN) and Mutchler, Inc. (Harrington Park, NJ), respectively. Deionized water was prepared on site. Blank rat plasma and milk were acquired from Bioreclamation (East Meadow, NY).

**Animals.** A total of 245 sexually mature, virgin female Crl:CD(SD) rats, approximately 70 days 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 g, 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 (National Research Council, 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 ± 5°F and 50 ± 20% relative humidity, 12-h light/dark photoperiod, and, at a minimum, 10 fresh air changes per h.

**Experimental Design.** Plasma concentrations after oral (gavage) administration to Sprague-Dawley Crl:CD(SD) dams or pups using one of two vehicles, CO or SPL, and single or multiple doses were investigated from 0 to 24 h after 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 in the DecaBDE groups) 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, whereas 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 were 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 h), and 0.5, 1, 2, 4, or 8 h after dosing. Milk collection occurred at 0.5, 1, 2, 4, 8, and 24 h after 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 18 naive 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). Blood was collected at sacrifice on PND 4 at 0.5, 1, 2, 4, 8, or 24 h after 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

TABLE 1

*Description of the DecaBDE dosage regimens, sample types collected, and sampling intervals in $F_0$ females, fetuses, and/or neonates*

| Group | Dosage Level | Vehicle | $F_0$ Females; Multiple Doses | | | | Naive Litters[a] (8 Pups/Litter); Single Direct Dose to Pups on PND 4 | |
|---|---|---|---|---|---|---|---|---|
| | | | GDs 6 through 20[b] | | GD 6 through LD 4[c] | | | |
| | | | No. Females | GD 20 Samples[d] | No. Females | LD 4, PND 4 Samples[e] | No. Pups | PND 4 Sample |
| | *mg/(kg · day)* | | | | | | | |
| 1 | 0 | CO | 4[f] | Blood | 10[f] | Milk and blood; blood | —[g] | — |
| 2 | 100 | CO | 24 | Blood | 24 | Milk and blood; blood | — | — |
| 3 | 300 | CO | 24 | Blood | 24 | Milk and blood; blood | — | — |
| 4 | 1000 | CO | 24 | Blood | 24 | Milk and blood; blood | — | — |
| 5 | 20 | CO | — | — | — | — | 18 | Blood |
| 6 | 20 | SPL | — | — | — | — | 18 | Blood |
| 7 | 0 | SPL | — | — | 10[d] | Milk and blood; blood | — | — |
| 8 | 1000 | SPL | — | — | 24 | Milk and blood; blood | — | — |
| A1[h] | 1 | CO | — | — | 3–4 | Milk and blood; blood | — | — |
| A2[h] | 10 | CO | — | — | 3–4 | Milk and blood; blood | — | — |

[a] Not previously exposed to DecaBDE.
[b] Samples were collected at 0 (predose) and at 0.5, 1, 2, 4, and 8 h after dosing on each day of sampling, except as indicated for controls.
[c] Samples were collected at 0.5, 1, 2, 4, 8, and 24 h after dosing on each day of sampling, except as indicated for controls.
[d] $F_0$ females and fetuses (GD 20).
[e] $F_0$ females (LD 4) and pups (PND 4).
[f] Blood collected only at 1 h.
[g] —, not performed.
[h] A, adjunct; samples collected at 8 h after dosing only on LD 4/PND 4.

mg/(kg · day), administered on GD 6 to LD 4, were determined in three or four $F_0$ females per group and their litters 8 h after dosing on LD 4.

Body weights and food consumption of the dams were measured on GDs 0, 6, 9, 12, 15, 18, and 20 and on LDs 1, 4, 7, 11, 14, 17, 21, and 22. Pups were individually weighed on PNDs 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 was 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 h before milk collection, and oxytocin, 1 IU (0.05 ml) subcutaneously, was administered to stimulate milk letdown. Milk was collected from the same dams as used for blood collection (four dams per group per time point; vehicle control rats were sampled at 4 h after dosing only). The mammary glands were cleaned, and milk was collected using gentle massage over the glands until sufficient sample size 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 (6890N; Agilent Technologies, Santa Clara, CA)-mass spectrometry (5975C; Agilent) with use of an electron ionization source. The method was validated for 100-$\mu$l sample volumes before use over the concentration ranges of 1000 through 6000 and 250 through 6000 ng of 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 quality control samples and at least one third at each level to be within 85 to 115% of the target quality control concentration. Pharmacokinetic parameters were analyzed using WinNonlin 5.2 Professional (Pharsight Corp., Mountain View, CA). Details on the materials and methods, statistics, and additional endpoints evaluated in the study are available in the supplemental data.

## Results

**Mortality, Morbidity, Body Weights, Food Consumption, and Gestational and Litter Data.** No test substance-related deaths or clinical signs of toxicity occurred in the $F_0$ females, when CO was the vehicle used to deliver the test substance. In addition, no test substance-related differences in mean body weight, body weight gain, or food consumption were detected during gestation or lactation (Supplemental Tables S1–S3). When SPL was the vehicle, mean food consumption by maternal animals was increased in the 1000-mg/(kg · day) group compared with 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) (Supplemental Table S3). Food consumption for the SPL 1000-mg/(kg · day) group was similar to that of the CO control over these intervals (Supplemental Table S3), as was body weight gain (Supplemental Tables S1 and S2).

DecaBDE administration in CO or SPL did not affect gestation or litter parameters (Supplemental Table S4). Mean gestational length in treated animals was comparable with that of 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 control and/or laboratory historical control means (Table 2; Supplemental Table S5). Body weights of male and female pups in the SPL 1000-mg/(kg · day) group were higher than those in their respective SPL control group on PNDs 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 those 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 GD 20.** DecaBDE plasma concentrations in dams and their litters just before and up to 8 h after dosing on GD 20 with CO as the vehicle are shown in Fig. 1 (Supplemental Table S6). The dams' mean plasma concentrations were similar at each time point regardless of dose [100, 300, or 1000 mg/(kg · day)]. Furthermore, DecaBDE concentrations were relatively constant, regardless of dose, over the entire sampling interval. A similar lack of dose and time response was seen in fetal plasma, but levels were typically 2.5- to 5-fold lower than those of the dams (Table 3).

TABLE 2

*$F_1$ pup body weights on PNDs 1 through 21*

$F_1$ pups derived from females administered DecaBDE at doses of 100 to 1000 mg/(kg · day) in CO or 1000 mg/(kg · day) in SPL from GD 6 through LD 22. Data are means ± S.E.M.

| PND | Body Weight with Maternal Dose (GD 6 through LD 21) and Vehicle | | | | | |
|---|---|---|---|---|---|---|
| | CO ($n$ = 9)[a] | CO 100 ($n$ = 22) | CO 300 ($n$ = 24) | CO 1000 ($n$ = 22) | SPL ($n$ = 10) | SPL 1000 ($n$ = 22) |
| | | | | *g* | | |
| $F_1$ males | | | | | | |
| 1 | 7.6 ± 0.27 | 7.5 ± 0.14 | 7.3 ± 0.10 | 7.3 ± 0.14 ($n$ = 23) | 6.7 ± 0.15 | 6.8 ± 0.13 ($n$ = 23) |
| 4 | 10.3 ± 0.48 | 10.4 ± 0.23 | 9.9 ± 0.19 | 9.8 ± 0.27 ($n$ = 23) | 8.0 ± 0.27 | 8.6 ± 0.20 ($n$ = 23) |
| 7 | 16.9 ± 0.90 | 16.9 ± 0.34 | 16.2 ± 0.32 | 15.9 ± 0.48 | 11.5 ± 0.73 | 13.5 ± 0.42* |
| 11 | 26.6 ± 1.27 | 26.6 ± 0.61 | 25.7 ± 0.45 | 25.5 ± 0.61 | 19.2 ± 1.45 | 21.9 ± 0.65 |
| 14 | 33.6 ± 1.51 | 33.4 ± 0.79 | 31.9 ± 0.54 | 31.4 ± 0.68 | 25.8 ± 1.66 | 29.2 ± 0.74* |
| 17 | 41.1 ± 1.79 | 39.4 ± 0.93 | 38.1 ± 0.66 | 38.8 ± 0.73 | 32.9 ± 1.86 | 36.0 ± 0.90 |
| 21 | 51.6 ± 2.25 | 50.7 ± 1.19 | 48.8 ± 0.74 | 48.1 ± 1.15 | 41.9 ± 2.53 | 47.6 ± 0.84* |
| $F_1$ females | | | | | | |
| 1 | 7.1 ± 0.27 | 7.1 ± 0.12 | 6.9 ± 0.10 | 7.0 ± 0.15 ($n$ = 23) | 6.2 ± 0.09 | 6.4 ± 0.12 |
| 4 | 9.6 ± 0.54 | 9.6 ± 0.23 | 9.4 ± 0.16 | 9.3 ± 0.25 ($n$ = 23) | 7.5 ± 0.24 | 8.0 ± 0.18 |
| 7 | 16.0 ± 0.86 | 15.8 ± 0.27 | 15.3 ± 0.32 | 15.2 ± 0.42 | 10.5 ± 0.59 | 12.3 ± 0.35* |
| 11 | 25.6 ± 1.39 | 25.1 ± 0.56 | 24.4 ± 0.53 | 24.7 ± 0.48 | 17.7 ± 1.16 | 20.3 ± 0.58* |
| 14 | 32.1 ± 1.87 | 32.2 ± 0.67 | 30.3 ± 0.61 | 30.9 ± 0.58 | 24.2 ± 1.43 | 27.4 ± 0.76* |
| 17 | 39.0 ± 1.99 | 38.3 ± 0.79 | 36.4 ± 0.75 | 37.6 ± 0.66 | 31.5 ± 1.74 | 34.1 ± 0.84 |
| 21 | 49.2 ± 2.51 | 49.2 ± 1.03 | 46.6 ± 0.95 | 47.2 ± 0.94 | 39.5 ± 2.08 | 44.4 ± 0.98* |

[a] Number of litters.
* Significantly different from SPL control ($p < 0.05$).

**Plasma and Milk Concentrations in Dams on LD 4.** Plasma concentrations in dams at 0.5 to 24 h after dosing on LD 4 after administration in CO over GD 6 through LD 4 are shown in Fig. 2 (Supplemental Tables S7 and 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). The $AUC_{last}$ for the CO test substance groups were similar on LD 4 across a 10-fold dose range. 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 that in CO (Fig. 3; Table 3).

Mean milk concentrations at doses of 100 to 1000 mg/(kg · day) in CO were relatively stable between 0.5 and 8 h after dosing, and were similar irrespective of dose (Fig. 4; 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 (Supplemental 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 h after dosing in the DNT study from dams administered DecaBDE in CO at 1 or 10 mg/(kg · day) from GD 6 through LD 4 (Supplemental Tables S7 and 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 in the 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.

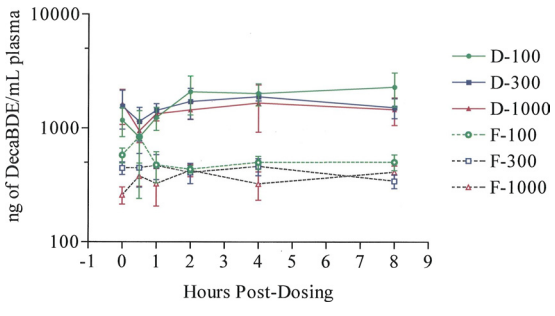


FIG. 1. DecaBDE plasma concentrations (nanograms per milliliter) on GD 20 in $F_0$ dams (D) and fetuses (F) after administration of DecaBDE in CO at 100, 300, and 1000 mg/(kg · day) to $F_0$ dams from GDs 6 through 20. Mean ± S.D.

**Plasma Concentrations in Corresponding PND 4 Pups.** The PND 4 mean plasma concentrations in the CO 100 to 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 (Fig. 2; Table 3; Supplemental Table S7). Pup plasma concentrations and $AUC_{last}$ were roughly 2-fold higher than those 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 (Fig. 3). However, the PND 4 pups mean plasma concentrations in the SPL 1000 mg/(kg · day) group were clearly (approximately 3-fold) lower than those in the CO 1000 mg/(kg · day) group (Supplemental Table S7).

PND 4 plasma samples were collected from pups at 8 h after dosing of the maternal females with CO at doses of 1 or 10 mg/(kg · day) in the DNT study (Supplemental 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 (Supplemental Table S7).

**Plasma Concentrations in Pups on PND 4 after a Single Dose.** Plasma concentrations after a single 20 mg/kg dose in CO to pups on PND 4 increased with time after dosing from <LOQ to 1845 ± 323 ng/ml at 0.5 and 24 h after dosing, respectively (Supplemental 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 $AUC_{0-8\,h}$ and the $AUC_{0-24\,h}$ were lower in directly dosed pups, compared with 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 supplemental data.

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*

| Parameter (units) | Maternal Plasma, GD 20 | | | Fetal Plasma, GD 20 | | | Maternal Plasma, LD 4 | | | Maternal Milk, LD 4 | | | Pup Plasma, PND 4 | | | Pup Plasma, PND4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CO 100 | CO 300 | CO 1000 | CO 100 | CO 300 | CO 1000 | CO 100 | CO 300 | CO 1000 | SPL 1000 | CO 100 | CO 300 | CO 1000 | SPL 1000 | CO 100 | CO 300 | CO 1000 | SPL 1000 | CO 20 | SPL 20 |
| $AUC_{last}$ (h · ng/ml) | 15,393 | 13,299 | 11,974 | 4087 | 3371 | 2934 | 30,559 | 25,663 | 24,354 | 5649 | 13,435 | 11,955 | 14,188 | 896 | 51,154 | 46,372 | 46,578 | 14,254 | —[a] | — |
| $C_{avg}$ (ng/ml) | — | — | — | 432 | 315 | — | 1273 | 1069 | 1015 | 235 | 560 | 498 | 591 | 95.0 | 594 | 2131 | 1932 | 1941 | — | — |
| $C_{max}$ (ng/ml) | 2299 | 1885 | 1666 | 839 | 469 | 426 | 1812 | 1462 | 1334 | 371 | 851 | 800 | 824 | 183 | 789 | 2535 | 2103 | 2414 | 1845 | 293 |
| $C_{min}$ (ng/ml) | 832 | 1147 | 949 | 436 | 343 | 260 | 715 | 613 | 573 | 162 | 380 | 269 | 439 | 0.00 | 491 | 1578 | 1600 | 1485 | — | — |
| Fluctuation (%) | — | — | — | 93.2 | 39.9 | — | 86.2 | 79.4 | 75.0 | 88.7 | 84.1 | 107 | 65.1 | 192 | 50.1 | 44.9 | 26.1 | 47.9 | — | — |
| Dose norm $AUC_{last}$ | 154 | 44.3 | 12.0 | 40.9 | 11.2 | 2.93 | 306 | 85.5 | 24.4 | 5.65 | 134 | 39.9 | 14.2 | 0.896 | 512 | 155 | 46.6 | 14.3 | — | — |
| Dose norm $C_{avg}$ | — | — | — | 4.32 | 1.05 | — | 12.7 | 3.56 | 1.01 | 0.235 | 5.60 | 1.66 | 0.591 | 0.095 | 5.94 | 7.10 | 1.93 | 1.94 | — | — |
| $AUC_{0-8\ h}$ (h · ng/ml) | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 8444 | 466 |
| $AUC_{0-24\ h}$ (h · ng/ml) | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 34823 | 3451 |
| Dose norm $AUC_{0-8\ h}$ | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 422 | 23.3 |
| Dose norm $C_{max}$ | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 92.3 | 14.7 |

$AUC_{last}$, the area under the plasma compound concentration vs. time curve from nominal time 0 to the last time point with quantifiable plasma compound concentration ($T_{last}$). Calculated by linear trapezoidal summation using the equation $AUC_{last} = \Sigma (0.5 \times (y1 + y2) \times \Delta t)$, where y1 and y2 are successive plasma compound concentrations and $\Delta t$ is the sampling interval, in hours, between y1 and y2; $C_{avg}$, the average concentration in plasma at steady state. $C_{avg} = AUC\tau/\tau$. $AUC\tau$ = the area under the plasma compound concentration vs. time curve from nominal time 0 to $\tau$ ($\tau$ = dosing interval); $C_{max}$, $C_{min}$, the observed maximum (minimum) concentration in plasma determined directly from the data; fluctuation %, percent fluctuation from the average concentration in plasma at steady state. Fluctuation % = 100* ($C_{max} - C_{min}$)/$C_{avg}$; dose norm $AUC_{last}$, $C_{avg}$, $AUC_{0-8\ hr}$, $C_{max}$, dose normalized; $AUC_{last}$, $C_{avg}$, $AUC_{0-8\ hr}$, $C_{max}$ divided by the daily dose in mg/(kg · day).

—, not calculated.

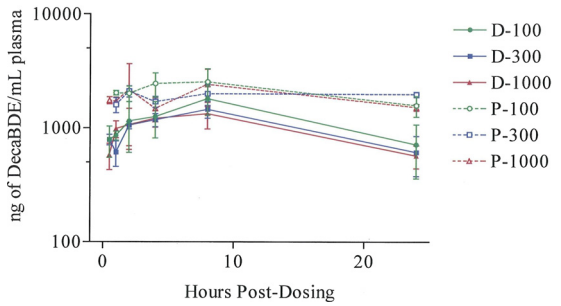


FIG. 2. DecaBDE plasma concentrations (nanograms per milliliter) on LD 4 in $F_0$ dams (D) and pups (P) after administration of DecaBDE in CO at 100, 300, and 1000 mg/(kg · day) to $F_0$ dams from GD 6 through LD 4. Mean ± S.D.

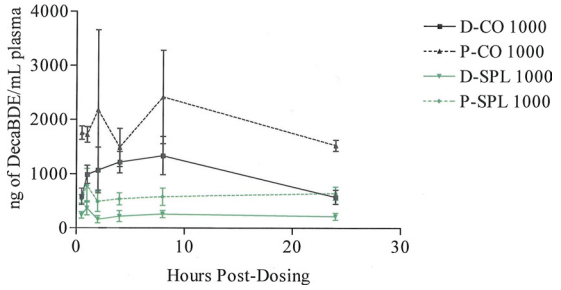


FIG. 3. Comparison of DecaBDE plasma concentrations in dams (D) and pups (P) on LD 4/PND 4 after administration of DecaBDE in CO or SPL at 1000 mg/(kg · day) to $F_0$ dams over GD 6 through LD 4. Mean ± S.D.

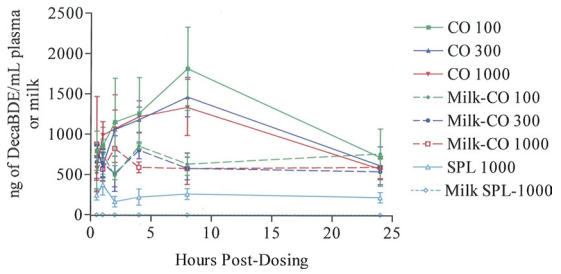


FIG. 4. Comparison of DecaBDE plasma and milk concentrations (nanograms per milliliter) on LD 4 of $F_0$ dams after administration in CO at 100, 300, and 1000 mg/(kg · day) or SPL at 1000 mg/(kg · day) over GD 6 through LD 4. Mean ± S.D.

## Discussion

Several studies have reported limited absorption of DecaBDE after oral administration (National Toxicology Program, 1986; el Dareer et al., 1987; Huwe et al., 2008). For example, the U.S. National Toxicology Program reported dietary absorption of approximately 0.33% of the dose by rats. However, 10% absorption from a single gavage dose, when SPL was used 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 higher solubility of DecaBDE in SPL, compared with two other vehicles, was demonstrated. The 10% value was subsequently interpreted as representing systemic absorption (i.e., bioavailability) without taking into account the negligible blood levels reported by Mörck et al. (2003) and others. Also not taken into consideration was the likelihood of extensive first-pass elimination, as suggested by the concentrations in bile versus blood. However, based on these data, the European Union was interested in use of SPL 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 in which doses up to 1000 mg/(kg · day) were administered from GDs 0 through 20 (Hardy et al., 2002).

Although not compared statistically, visual inspection of the data showed that mean body weights in lactating SPL control dams were typically lower than those in CO administered dams. Food consumption in the SPL control dams was also lower than CO control or CO 1000 mg/(kg · day) dams, whereas food consumption in the SPL 1000 mg/(kg · day) dams approached that of the dams treated with a test substance using CO. Furthermore, mean body weights of pups derived from dams administered repeated doses of the SPL vehicle were lower than those of pups derived from the 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 to be 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 administered to dams in SPL over GD 6 through LD 4. Likewise, DecaBDE plasma concentrations were lower in naive 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.

With CO as the vehicle, DecaBDE plasma concentrations in dams and fetal litters appeared to be at steady-state levels when first sampled on GD 20; plasma concentrations were similar at the first (immediately before dosing) and last (8 h) collection times after 15 consecutive doses. Steady-state was also apparent in dam and neonatal pup plasma concentrations measured on LD 4 after dosing from GD 6 to LD 4. Steady-state was likely reached within a few days of dose initiation, but blood samples were first collected after 15 daily doses. Attainment of steady-state levels during this time frame is consistent with elimination of >99% of a dietary dose within 72 h (National Toxicology Program, 1986), as well as the 2008 report of Huwe et al. noting that 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 five to seven half-lives, the results of Huwe et al. (2008) further suggest that adipose half-lives of 0.4 to 2.8 days are likely for these 10 lower brominated congeners. Although a component in the test mixture administered in the study of Huwe et al. (2008), BDE 209 was not included in the 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 times than other tissues. When viewed in total, our data along with other reports (Norris et al., 1973, 1974, 1975; el Dareer et al., 1987) demonstrate that 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.

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 2 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, such that only a small fraction of the dose is bioavailable.

DecaBDE is expected to bind to particulates, including those found in fecal matter, and evidence for this is seen in studies reporting a substantial fraction of the dose could not be extracted from the feces (National Toxicology Program, 1986; el Dareer et al., 1987; Mörck et al., 2003; Huwe and Smith, 2007; Huwe et al., 2008). However, the binding properties of DecaBDE have led to different conclusions regarding its fate in the body. For example, Huwe and Smith (2007) reported nonextraction 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). Likewise, Mörck et al. (2003) concluded that 65% of an oral dose of [$^{14}$C]BDE-209 was present in the gut as metabolites. These authors defined metabolites as all $^{14}$C activity not extracted with the parent molecule, including that engaged in nonspecific binding. However, other studies using $^{14}$C-labeled test articles have demonstrated that after oral administration, DecaBDE is poorly metabolized and predominantly excreted in the feces as parent molecule (National Toxicology Program, 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) probably imparts a negative effect on its permeability, as does the molecule's noncoplanar 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 (National Toxicology Program, 1986). Furthermore, 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) probably governed absorption at the dose of 1 mg/(kg · day). Figure 1 of Sandholm et al. (2003) suggests first-order absorption kinetics at a dose of approximately 2 mg/kg based on the similar terminal slopes of the oral and intravenous concentration-time curves. 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 suggests that 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 those of 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 with the plasma concentrations in dams suggests, as expected because of 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. In addition, for the first time, maximal DecaBDE 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.

## References


Coffee ST (2008) Development and validation of a GC-ECD assay for the determination of decabromodiphenyl oxide (Debdpo) concentration in corn oil (Study No. WIL-635004), WIL Research Laboratories, LLC, Ashland, OH.

European Commission (2002) EUR 20402 EN—European Union risk assessment report bis(pentabromophenyl) ether, in *1st Priority List* (Hansen BG, Munn SJ, de Bruijn J, Luotamo M, Pakalin S, Berthault F, Vegro S, Pellegrini G, Allanou R, and Scheer S eds) pp 1–282, Office for Official Publications of the European Communities, Luxembourg.

European Commission (2006) Commission Regulation (EC) No 565/2006 of 6 April 2006 imposing testing and information requirements on the importers or manufacturers of certain priority substances in accordance with Council Regulation (EEC) No 793/93 on the evaluation and control of the risks of existing substances. *OJ* **L99:**3–5.

el Dareer SM, Kalin JR, Tillery KF, and Hill DL (1987) Disposition of decabromobiphenyl ether in rats dosed intravenously or by feeding. *J Toxicol Environ Health* **22:**405–415.

Hardy M, Banasik M, and Stedeford T (2009) Toxicology and human health assessment of decabromodiphenyl ether. *Crit Rev Toxicol* **39 (Suppl 3):**1–44.

Hardy ML (2002) The toxicology of the three commercial polybrominated diphenyl oxide (ether) flame retardants. *Chemosphere* **46:**757–777.

Hardy ML, Schroeder R, Biesemeier J, and Manor O (2002) Prenatal oral (gavage) developmental toxicity study of decabromodiphenyl oxide in rats. *Int J Toxicol* **21:**83–91.

Huwe JK, Hakk H, Smith DJ, Diliberto JJ, Richardson V, Stapleton HM, and Birnbaum LS (2008) Comparative absorption and bioaccumulation of polybrominated diphenyl ethers following ingestion via dust and oil in male rats. *Environ Sci Technol* **42:**2694–2700.

Huwe JK and Smith DJ (2007) Accumulation, whole-body depletion, and debromination of decabromodiphenyl ether in male Sprague-Dawley rats following dietary exposure [published erratum appears in *Environ Sci Technol* **41:** 4485, 2007]. *Environ Sci Technol* **41:**2371–2377.

Kociba RJ, Frauson LO, Humiston CG, Norris JM, Wade CE, Lisowe RW, Quast JF, Jersey GC, and Jewett GL (1975) Results of a two-year dietary feeding study with decabromodiphenyl oxide (DBDPO) in rats. *J Fire Flammability/Combustion Toxicol* **2:**267–285.

Morck A, Hakk H, Orn U, and Klasson Wehler E (2003) Decabromodiphenyl ether in the rat: absorption, distribution, metabolism, and excretion. *Drug Metab Dispos* **31:**900–907.

Mörck A and Klasson-Wehler E (2001) Metabolism of decabromodiphenyl ether (BDE-209) in the rat, in *Second International Workshop on Brominated Flame Retardants*, pp 291–294, Stockholm University, Stockholm, Sweden.

Norris JM, Ehrmantraut JW, Gibbons CL, Kociba RJ, Schwetz BA, Rose JQ, Humiston CG, Jewett GL, Crummett WB, Gehring PJ, et al. (1973) Toxicological and environmental factors involved in the selection of decabromodiphenyl oxide as a fire retardant chemical. *Appl Polym Symp* **22:**195–219.

Norris JM, Ehrmantraut JW, Gibbons CL, Kociba RJ, Schwetz BA, Rose JQ, Humiston CG, Jewett GL, Crummett WB, Gehring PJ, et al. (1974) Toxicological and environmental factors involved in the selection of decabromodiphenyl oxide as a fire retardant chemical. *JFF/Combustion Toxicol* **1:**52–77.

Norris JM, Kociba RJ, Schwetz BA, Rose JQ, Humiston CG, Jewett GL, Gehring PJ, and Mailhes JB (1975) Toxicology of octabromobiphenyl and decabromodiphenyl oxide. *Environ Health Perspect* **11:**153–161.

National Research Council (1996) *Guide for the Care and Use of Laboratory Animals*, 7th ed. National Academy Press, Washington, DC.

National Research Council (2000) Decabromodiphenyl oxide, in *Toxicological Risks of Selected Flame-Retardant Chemicals*, pp 72–98, National Academy Press, Washington, DC.

National Toxicology Program (1986) Toxicology and carcinogenesis studies of decabromodiphenyl oxide (CAS No. 1163–19-5) in F344/N rats and B6C3F1 mice (feed studies). Technical Report Series 309, pp 1–242.

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.

Organisation for Economic Co-operation and Development (1997) OECD Principles on good laboratory practice [C(97)186/Final], Organisation for Economic Co-operation and Development, Paris, France.

Organisation for Economic Co-operation and Development (2007) OECD guideline for the testing of chemicals. Developmental neurotoxicity study, pp 1–26, Organisation for Economic Co-operation and Development, Paris, France.

Riu A, Cravedi JP, Debrauwer L, Garcia A, Canlet C, Jouanin I, and Zalko D (2008) Disposition and metabolic profiling of [$^{14}$C]-decabromodiphenyl ether in pregnant Wistar rats. *Environ Int* **34:**318–329.

Sandholm A, Emanuelsson BM, and Wehler EK (2003) Bioavailability and half-life of decabromodiphenyl ether (BDE-209) in rat. *Xenobiotica* **33:**1149–1158.

van de Waterbeemd H and Testa B (2009) Introduction: the why and how of drug bioavailability research, in *Drug Bioavailability—Estimation of Solubility, Permeability, Absorption and Bioavailability*, 2nd, completely revised edition (van de Waterbeemd H and Testa B eds), pp 1–6, Wiley-VCH Verlag GmbH & Co., Weinheim, Germany.

Viberg H, Fredriksson A, Jakobsson E, Orn U, and Eriksson P (2001) Brominated flame 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, Stockholm, Sweden.

Viberg H, Fredriksson A, Jakobsson E, Orn U, and Eriksson P (2003) Neurobehavioral derangements in adult mice receiving decabrominated diphenyl ether (PBDE 209) during a defined period of neonatal brain development. *Toxicol Sci* **76:**112–120.

Zhou T, Ross DG, DeVito MJ, and Crofton KM (2001) Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicol Sci* **61:**76–82.

Zoetis T and Walls I (2003) *Principles and Practices for Direct Dosing of Pre-Weaning Mammals in Toxicity Testing and Research. A Report of the ILSI Risk Science Institute Expert Working Group*, ILSI Press, Washington, DC.


**Address correspondence to:** John A. Biesemeier, Corporate Senior Toxicologist, Chemtura Corporation, 1801 U.S. Highway 52 W, West Lafayette, IN 47906. E-mail: john.biesemeier@chemtura.com