

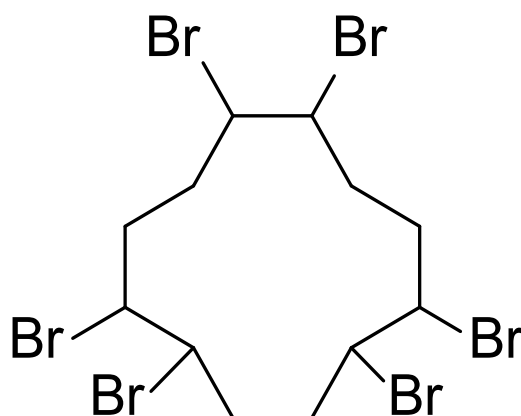


United States
Environmental Protection Agency

Office of Chemical Safety and
Pollution Prevention

Draft Risk Evaluation for Cyclic Aliphatic Bromides Cluster (HBCD)

Supplemental Information on Human Health Hazard



CASRN	NAME
25637-99-4	Hexabromocyclododecane
3194-55-6	1,2,5,6,9,10-Hexabromocyclododecane
3194-57-8	1,2,5,6-Tetrabromocyclooctane

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1 Detailed Hazard Overview

1.1 Thyroid Effects

1.1.1 Human Evidence

The association between HBCD exposure and alterations of thyroid hormones was investigated in populations at different lifestages. Specifically, investigations of the potential effects of HBCD on the thyroid in humans have been conducted in infants and children participating in birth cohort studies in the Netherlands ([Roze et al., 2009](#)) and Norway ([Eggesbø et al., 2011](#)), adolescents participating in a cross-sectional general population study in areas around industrial sites in Belgium ([Kiciński et al., 2012](#)), and adult men attending an infertility clinic in the United States (cross-sectional study) ([Johnson et al., 2013](#)). In addition, there is one case-control study of hypothyroidism in Korean mother and infant pairs ([Kim and Oh, 2014](#)). Of these five studies, only two were large scale (>500 participants) ([Kiciński et al., 2012](#); [Eggesbø et al., 2011](#)), and only one included an analysis that allowed for the examination of exposure-response patterns ([Eggesbø et al., 2011](#)). Quantitative methods used by several of the studies resulted in 25–75% of samples below stated detection limits ([Kim and Oh, 2014](#); [Kiciński et al., 2012](#); [Eggesbø et al., 2011](#)). While some of the available studies included consideration of other suspected thyroid-disrupting chemicals, none considered known thyroid antagonists such as perchlorate, thiocyanate, or nitrate ([Steinmaus et al., 2013](#); [Tonacchera et al., 2004](#)). Other study limitations and a summary of overall confidence in the results are noted in Table 1-1. Studies are ordered by the age at outcome evaluation, and then by overall confidence in the study.

A Norwegian birth cohort did not find a statistically significant association between the levels of HBCD measured in breast milk and thyroid-stimulating hormone (TSH) levels in newborns ([Eggesbø et al., 2011](#)). Elevated, but non-statistically significant, odds ratios (range: 1.3–1.6) were reported for increased TSH in relation to increasing HBCD levels in breast milk that are suggestive of a potential association; however, confidence intervals (CIs) around each of the point estimates were relatively wide (based on approximately 30 individuals per group) and a clear dose-response was not observed. This analysis controlled for several potential mediators of normal thyroid hormone variability and several thyroid disruptors (e.g., polychlorinated biphenyls [PCBs], polybrominated diphenyl ethers [PBDEs], and hexachlorobenzene). Adjustments for iodine deficiency were not made; however, the study authors noted that this condition is rare in Norway ([Eggesbø et al., 2011](#)).

A study in adolescents ages 13–17 years who lived in areas around industrial sites in Belgium (n = 515) did not find an association between serum concentrations of HBCD and concurrent measures of TSH, thyroxine (T4), or triiodothyronine (T3) ([Kiciński et al., 2012](#)). Since approximately 75% of serum concentrations were below the limit of quantitation (LOQ), analyses were dichotomized to compare effects associated with HBCD concentrations above and below the LOQ. The three remaining studies ([Kim and Oh, 2014](#); [Johnson et al., 2013](#); [Roze et al., 2009](#)) had reporting deficiencies that limit the ability to interpret results from these studies (Table 1-2). In studies of infants ([Roze et al., 2009](#)) and adult men ([Johnson et al., 2013](#)), the

authors did not identify a statistically significant relationship between HBCD and a specific thyroid hormone; quantitative results pertaining to the magnitude or direction of association between HBCD and thyroid hormones were not reported. [Kim and Oh \(2014\)](#) found no significant correlations between α -, β -, or γ -HBCD and any thyroid hormones in infants with congenital hypothyroidism; however, reporting limitations of this case-control study (i.e., no information on participant recruitment) and analysis (i.e., 25% of samples were below the limit of detection [LOD]) were noted.

The human database for HBCD is inadequate to support conclusions regarding the relationship between HBCD exposure and thyroid effects. The studies of HBCD exposure in relation to variation in thyroid hormone levels or thyroid disease (congenital hypothyroidism) do not provide a basis for assessing a causal association at any lifestage.

1.1.2 Animal Evidence

Several short-term and subchronic rodent studies evaluated the effects of HBCD on the thyroid, specifically serum thyroid hormone levels, thyroid histopathology, and thyroid weight. Two of these studies investigated thyroid-related endpoints at time-points approximately 4–8 weeks following the end of dosing ([Saegusa et al., 2009](#); [WIL Research, 2001](#)). The evidence pertaining to thyroid effects in experimental animals following oral exposure to HBCD is summarized in Table 1-2 and Figure 1-1. Exposure response array of thyroid effects following oral exposure. Effect categories with stronger evidence are presented first, with individual studies ordered by study duration and then species. If not otherwise indicated, endpoint measurements were made in adults.

1.1.3 Thyroid Hormones

Several studies in rats reported HBCD-related effects on thyroid hormone levels using radioimmunoassay ([van der Ven et al., 2009](#); [Ema et al., 2008](#); [van der Ven et al., 2006](#)) or electrochemiluminescence immunoassay ([Saegusa et al., 2009](#); [WIL Research, 2001](#)).

TSH levels were generally increased in most dosed groups (male and female F0 and F1 CD rats ([Ema et al., 2008](#)), male and female CD rats ([WIL Research, 2001](#)), and male weanling CD rats ([Saegusa et al., 2009](#)). These increases reached statistical significance in male weanlings (postnatal day [PND] 20) ([Saegusa et al., 2009](#)) and female adult rats (F0 and F1) ([Ema et al., 2008](#)). Additional support for HBCD-mediated increases in TSH are provided by [van der Ven et al. \(2006\)](#); although serum TSH levels were not directly measured, female rats exposed to 200 mg/kg-day HBCD for 28 days showed a statistically significant increase in pituitary TSH immunostaining, suggesting elevated synthesis and release of this hormone.

Statistically significant decreases in T4 (up to –38% of control) were observed in F0 rats exposed to approximately 1,000–1,300 mg/kg-day HBCD ([Ema et al., 2008](#)). A dose-related decrease in T4 was also observed in the F1 generation, with a 28% decrease in T4 in high-dose females ([Ema et al., 2008](#)). Similarly, male and female rats exposed for 90 days to doses up to 1000 mg/kg-day were observed to have a dose-related decrease in T4 (up to -37% of control) ([WIL Research, 2001](#)). Adult female rats exposed to up to 200 mg/kg-day HBCD for 28 days also showed a significant dose-dependent decrease in serum T4 (26% decrease at 200 mg/kg-day) ([van der Ven et al., 2006](#)); a dose-related decrease was not observed in male rats in the same study. The available developmental and one-generation toxicity studies did not detect alterations

in levels of T4 in offspring at maternal doses ranging from approximately 100 to 1,500 mg/kg-day ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#)). Serum levels of T3 were also investigated in several studies ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#); [van der Ven et al., 2006](#); [WIL Research, 2001](#)), but only one detected a statistically significant effect. A 15% decrease in T3 levels relative to controls was observed in male weanling rats treated gestationally and lactationally at maternal doses of 1,505 mg/kg-day ([Saegusa et al., 2009](#)).

The pattern of increased TSH and decreased T4 observed in the two-generation reproductive study ([Ema et al., 2008](#)) is consistent with the multi-loop feedback system of the hypothalamus-pituitary-thyroid (HPT) axis ([Fisher and Nelson, 2012](#)). The same patterns of effect in TSH and T4 were reported by W.I.L Research ([2001](#)); however, confidence in the hormone measurements from this study is low because approximately 50% of control samples used for TSH measurements were below the limit of detection and the remaining samples were 1–2 orders of magnitude lower than controls in other available studies, calling into question the conduct of the assay.

Two studies also measured thyroid hormone levels 4 weeks ([WIL Research, 2001](#)) or 8 weeks ([Saegusa et al., 2009](#)) after the end of dosing. Treatment-related changes in TSH and T3 levels were still present 8 weeks after the end of dosing in developmentally-exposed rats; however, the change was statistically significant for T3 only ([Saegusa et al., 2009](#)). In contrast, T4 and TSH levels in rats exposed as adults returned to control levels within 4 weeks after cessation of exposure ([WIL Research, 2001](#)).

1.1.4 Thyroid Histopathology

Histopathological changes indicative of thyroid activation were observed in some studies in experimental animals following exposure to HBCD. A 28-day study using doses up to 200 mg/kg-day qualitatively reported a dose-dependent increase in thyroid activation (i.e., follicle size, epithelial cell height, vacuolization, and nuclear size) in both male and female adult rats ([van der Ven et al., 2006](#)). A dose-related increase in the incidence of thyroid follicular cell hypertrophy was reported in adult male and female rats exposed to HBCD for 90 days and in female rats developmentally exposed to approximately 1,000–1,500 mg/kg-day for 30 days ([Saegusa et al., 2009](#); [WIL Research, 2001](#)). A similar dose-related effect was not observed in a 28-day study at doses up to 1,000 mg/kg-day ([WIL Research, 1997](#)) or in a two-generation reproductive toxicity study at doses up to approximately 1,300 mg/kg-day ([Ema et al., 2008](#)). A statistically significant increase (46–87%) in the incidence of small thyroid follicles was reported in both F0 and F1 high-dose animals in a two-generation reproductive toxicity study ([Ema et al., 2008](#)). This histological observation is likely indicative of a loss of colloid, which functions as a reservoir from which T3 and T4 can be released into the bloodstream as needed. With long-term TSH elevation, endocytosis of colloid occurs faster than synthesis, resulting in the progressive depletion of colloid and decreased follicle size ([Rosol et al., 2013](#)). Female mice exposed to approximately 200 mg/kg-day HBCD for 28 days showed a 20 and 26% decrease in follicle and colloid areas, respectively; however, this change did not reach statistical significance ([Maranghi et al., 2013](#)).

1.1.5 Thyroid Weight

Several studies in rats reported treatment-related increases in thyroid weight ([Saegusa et al., 2009](#); [Ema et al., 2008](#); [van der Ven et al., 2006](#); [WIL Research, 2001](#)); however, the response

patterns were not consistently dose-related nor were responses consistent across sexes. In animals exposed as adults only, several studies reported increased relative thyroid weights in female rats at doses ranging from approximately 30 to 1,500 mg/kg-day HBCD (Saegusa et al., 2009; Ema et al., 2008; van der Ven et al., 2006; WIL Research, 2001), whereas only one study reported the same effect in males exposed to approximately 1,000 mg/kg-day (Ema et al., 2008). In animals exposed to HBCD during development, statistically significant increases in thyroid weight were observed in male and female F1 adults exposed to 1,142 and 1,363 mg/kg-day, respectively (Ema et al., 2008) and adult males, but not females, 8 weeks after gestational and lactational exposure to ≥ 146 mg/kg-day (Saegusa et al., 2009). In a one-generation reproductive study, no changes in absolute thyroid weight were reported in male or female F1 rats at doses up to 100 mg/kg-day (van der Ven et al., 2009); relative thyroid weight was not reported.

Table 1-1. Evidence pertaining to thyroid effects in humans following exposure to HBCD

Reference and study design	Results		
<i>Studies in infants</i>			
<p>Eggesbø et al. (2011) (Norway, 2003–2006) Population: Birth cohort, recruited within 2 wks of delivery (able and willing to provide breast milk sample), 396 randomly selected for analysis; 239 of these were after February 2004 when the link to the thyroid screening data became available; 193 with HBCD data (46% girls) Exposure measures: Breast milk, collected at a median of 33 d after delivery (samples pooled over 8 consecutive mornings) Total HBCD detected in 67.9% of samples LOQ = 0.2 ng/g lipid Median 0.54 (range: 0.1–31) ng/g lipid Effect measures: TSH (whole blood spots) measured in infants 3 d after delivery (linked data beginning in February 2004); immunoassay (clinical lab) Analysis: Linear regression for ln TSH (continuous) and logistic regression for dichotomized ln TSH (at 80th percentile); see results column for consideration of covariates. Referent category includes all samples less than the LOQ (n = 62, 32%); remainder of population divided into four equally-sized categories. Data Quality: High (1.4)</p>	Association between HBCD level in breast milk with neonatal TSH levels:		
	Exposure category (ng/g lipid) (N)	Adjusted beta for ln TSH (95% CI) ^b	Adjusted odds ratio for TSH $\geq 80^{\text{th}}$ percentile (95% CI) ^c
	0.10 (62)	<i>(Referent)</i>	<i>(Referent)</i>
	0.13–0.52 (31)	–0.01 (–0.21, 0.20)	1.3 (0.3, 4.5)
	0.53–0.79 (33)	0.02 (–0.18, 0.22)	1.4 (0.3, 6.1)
	0.80–1.24 (33)	0.12 (–0.08, 0.33)	1.6 (0.4, 6.1)
	1.29–31.2 (34)	0.03 (–0.17, 0.23)	1.3 (0.3, 5.8)
Per interquartile range increase:	–0.00 (–0.02, 0.02)	1.0 (0.8, 1.1)	
Adjusted for age at TSH screening, maternal BMI, county, p,p-DDE, hexachlorobenzene, delivery type, pregnancy preeclampsia, and hypertension. Also evaluated but eliminated were maternal education, age at delivery, Norwegian nationality, season, parity, smoking, sex, gestational age, beta-hexachlorocyclohexane, oxychlorodane, and sum of all PCB congeners.			
EPA has lower confidence in results per interquartile range increase than in categorical analysis; this analysis used HBCD as a continuous variable. The inclusion of non-detects in this analysis presents considerable uncertainty in the interpretation of the results.			

Reference and study design	Results																											
<p>Roze et al. (2009) (the Netherlands, COMPARE cohort, 2001–2002) Population: Birth cohort, 90 singleton, term births, 62 of 69 (90%) mother-child pairs randomly selected from the cohort for HBCD measures in serum Exposure measures: Prenatal exposure, maternal serum at 35th week of pregnancy 1,2,5,6,9,10-HBCD (HBCD) detected in all samples LOD 0.8 pg/g serum Median 0.8 (range: 0.3–7.5) ng/g lipids Effect measures: Thyroid hormones (cord blood samples, n = 51, selected based on amount of sample available): T4, free T4, reverse T3, T3, TSH, throxine-binding globulin (assay not described) Analysis: Pearson correlation (for normally distributed variables) or Spearman’s rank correlation (for non-normally distributed variables) Data Quality:^a Medium (1.8)</p>	<p>Results for correlations between HBCD and cord blood thyroid hormone levels were not shown, but were stated to be not statistically significant.</p>																											
<p>Kim and Oh (2014) (South Korea, 2009–2010) Population: 26 infants with congenital hypothyroidism and their mothers, 12 healthy infant-mother pairs from the same hospital department also collected (case-control). Age of infants 1–24 mo; most 1–3 mo; excluded obese mothers (normal group only). Sex of infants not reported. Exposure measures: Serum, α, β, γ-HBCD, most samples collected 1–3 mo after birth, samples from two congenital hypothyroidism infants collected 18 and 24 mo after birth LOQ 0.036 ng/g lipid (% less than detection limit not reported) Total HBCD: Mean 8.55 ng/g lipid, range from less than method detection limit to 166 ng/g lipid Effect measures: Congenital hypothyroidism (not defined) Analysis: Two-sided student t-tests; comparisons between mothers of cases and controls, and between infant cases and controls. Values below LOQ replaced by a value of 0.5 times the LOQ; concentration data normalized, excluding outliers (not defined), to sum of PBDEs, HBCDs, and tetrabromobisphenol A. Data Quality:^a Medium (1.9)</p>	<table border="1"> <thead> <tr> <th></th> <th colspan="2">Congenital hypothyroidism Healthy controls</th> </tr> <tr> <th></th> <th colspan="2">Mothers, mean HBCD level (SD)</th> </tr> </thead> <tbody> <tr> <td>α-HBCD</td> <td>0.494 (1.52)</td> <td>2.57 (1.48)*</td> </tr> <tr> <td>β-HBCD</td> <td>0.27 (0.933)</td> <td>0.461 (1.08)</td> </tr> <tr> <td>γ-HBCD</td> <td>2.72 (1.42)</td> <td>8.86 (2.81)</td> </tr> <tr> <th></th> <th colspan="2">Infants, mean HBCD level (SD)</th> </tr> <tr> <td>α-HBCD</td> <td>2.42 (3.33)</td> <td>1.84 (2.5)</td> </tr> <tr> <td>β-HBCD</td> <td>0.578 (1.71)</td> <td>0.462 (0.768)</td> </tr> <tr> <td>γ-HBCD</td> <td>5.16 (2.42)</td> <td>14.05 (2.87)</td> </tr> </tbody> </table>		Congenital hypothyroidism Healthy controls			Mothers, mean HBCD level (SD)		α -HBCD	0.494 (1.52)	2.57 (1.48)*	β -HBCD	0.27 (0.933)	0.461 (1.08)	γ -HBCD	2.72 (1.42)	8.86 (2.81)		Infants, mean HBCD level (SD)		α -HBCD	2.42 (3.33)	1.84 (2.5)	β -HBCD	0.578 (1.71)	0.462 (0.768)	γ -HBCD	5.16 (2.42)	14.05 (2.87)
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<i>Studies in adolescents</i>																												
<p>Kiciński et al. (2012) (Belgium, 2008–2011) Population: 515 adolescents (13–17 yrs old) from two industrial sites and randomly selected from the</p>	<p>Thyroid hormone results (estimated from Figure 4 of Kiciński et al. (2012): Beta (95% CI)^d</p>																											

Reference and study design	Results						
<p>general population; participation rates 22–34% in the three groups, sample size varied by test</p> <p>Exposure measures: Serum samples, HBCD >75% were less than the LOQ (LOQ = 30 ng/L); Median <30 (range: <LOQ–234) ng/L</p> <p>Effect measures: Thyroid hormones: Free T3, free T4, TSH (immunoassay not described)</p> <p>Analysis: Regression models (linear or negative binomial depending on outcome); HBCD dichotomized</p> <p>Data Quality:^a Medium (1.9)</p>	<table border="0"> <tr> <td>Free T3 (pg/mL)</td> <td>0.08 (–0.08, 2.3)</td> </tr> <tr> <td>FreeT4 (mg/dL)</td> <td>–0.02 (–0.03, 0.09)</td> </tr> <tr> <td>TSH (%)</td> <td>0.0 (–4, 13)</td> </tr> </table> <p>Linear regression models for free T3 and free T4; negative binomial model for TSH. All models adjusted for age, gender, blood lipids, and BMI. Additional covariates evaluated included smoking, parental smoking, parental education, and parental home ownership, physical activity, computer use, alcohol and fish consumption, blood lead, and blood PCBs, and were included based on a stepwise regression procedure.</p>	Free T3 (pg/mL)	0.08 (–0.08, 2.3)	FreeT4 (mg/dL)	–0.02 (–0.03, 0.09)	TSH (%)	0.0 (–4, 13)
Free T3 (pg/mL)	0.08 (–0.08, 2.3)						
FreeT4 (mg/dL)	–0.02 (–0.03, 0.09)						
TSH (%)	0.0 (–4, 13)						
<i>Studies in adult men</i>							
<p>Johnson et al. (2013) (United States, 2002–2003)</p> <p>Population: 38 men (18–54 yrs old), from couples seeking infertility treatment; approximately 65% participation into general study; participation rate in the vacuum bag collection phase of the study not reported</p> <p>Exposure measures: HBCD exposure from vacuum bag dust; three main stereoisomers of HBCD presented together HBCD detected in 97% of samples; LOD not reported; median 246 ng/g dust (90th percentile 1,103 ng/g dust)</p> <p>Effect measures: Non-fasting blood sample immunoassay details in (Meeker et al., 2008) TSH free T4 free T3</p> <p>Analysis: All variables analyzed as continuous variables; Spearman’s correlation between HBCD in house dust and serum hormone levels; multivariable models adjusted for age and BMI</p> <p>Data Quality:^a High (1.6)</p>	<p>Adjustment for age and BMI produced similar results to the bivariate results (data not reported).</p> <p>No statistically significant changes in thyroid hormones (result not shown).</p>						

* $p = 0.004$; unadjusted for age and sex.

^aBased on OPPT data evaluation criteria

^b0.0 = no association.

^c1.0 = no association.

^dBeta is for HBCD >30 ng/L (LOQ) versus <30 ng/L; 0.0 = no association.

BMI = body mass index; EPA = U.S. Environmental Protection Agency; SD = standard deviation

Table 1-2. Evidence pertaining to thyroid effects in animals following exposure to HBCD

Reference and study design	Results				
<i>Serum thyroid hormones</i>					
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Thyroid hormones were measured by radioimmunoassay in adults only Data Quality: ^d High (1.0)	Doses (mg/kg-d)				
	Male, F0	0	10	101	1,008
	Female, F0	0	14	141	1,363
	Male, F1	0	11	115	1,142
	Female, F1	0	14	138	1,363
	TSH (ng/mL)				
	Male, F0 (n = 8)				
	Mean (SD)	16.15 (3.78)	16.18 (8.61)	19.14 (6.02)	23.26 (10.90)
	% of control ^a	–	0%	19%	44%
	Female, F0 (n = 8)				
	Mean (SD)	10.68 (1.35)	14.83* (2.47)	15.37* (2.17)	21.59* (8.87)
	% of control ^a	–	39%	44%	102%
	Male, F1 (n = 8)				
	Mean (SD)	11.93 (4.62)	11.50 (2.94)	15.78 (6.48)	15.54 (5.76)
	% of control ^a	–	–4%	32%	30%
	Female, F1 (n = 8)				
	Mean (SD)	10.35 (2.04)	15.36 (4.18)	18.09* (5.23)	17.28* (5.58)
	% of control ^a	–	48%	75%	67%
	T4 (µg/dL)				
	Male, F0 (n = 8)				
Mean (SD)	4.04 (1.42)	3.98 (0.89)	2.97 (0.76)	2.49* (0.59)	
% of control ^a	–	–1%	–26%	–38%	
Female, F0 (n = 8)					
Mean (SD)	2.84 (0.61)	3.14 (0.48)	3.00 (0.77)	1.96* (0.55)	
% of control ^a	–	11%	6%	–31%	
Male, F1 (n = 8)					
Mean (SD)	3.54 (0.29)	3.44 (0.86)	3.32 (0.98)	3.18 (0.48)	
% of control ^a	–	–3%	–6%	–10%	
Female, F1 (n = 8)					
Mean (SD)	3.59 (1.08)	3.56 (0.53)	3.39 (1.21)	2.58 (0.37)	
% of control ^a	–	–1%	–6%	–28%	
T3 (ng/dL)					
Male, F0 (n = 8)					
Mean (SD)	143.6 (29.0)	138.2 (21.6)	121.6 (15.6)	126.9 (16.3)	
% of control ^a	–	–4%	–15%	–12%	
Female, F0 (n = 8)					
Mean (SD)	133.1 (15.9)	140.9 (16.3)	146.5 (29.5)	134.7 (25.6)	
% of control ^a	–	6%	10%	1%	
Male, F1 (n = 8)					

Reference and study design	Results								
	Mean (SD)	122.1 (9.9)	123 (13.7)	123.6 (22.6)	122.3 (20.4)				
	% of control ^a	-	1%	1%	0%				
	Female, F1 (n = 8)								
	Mean (SD)	146.7 (17.5)	143.3 (18.1)	132.1 (26.2)	130.4 (17.8)				
<p data-bbox="190 464 483 541">van der Ven et al. (2009)</p> <p data-bbox="190 541 483 636">Rats, Wistar Diet One generation</p> <p data-bbox="190 636 483 972">F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11</p> <p data-bbox="190 972 483 1119">Thyroid hormones (total T3/T4) were measured by radioimmunoassay in adults only</p> <p data-bbox="190 1119 483 1213">Data Quality:^d High (1.2)</p>	Doses (mg/kg-d)								
		0	0.1	0.3	1	3	10	30	100
	T4 (nmol/L)								
	Male, F0 (n = 5)^b								
	Mean (SD)	62.0 (4.7)	-	-	-	-	-	-	54.2 (13.8)
	% of control ^a	-	-	-	-	-	-	-	-13%
	Female, F0 (n = 5)^b								
	Mean (SD)	44.4 (9.3)	-	-	-	-	-	-	38.0 (17.6)
	% of control ^a	-	-	-	-	-	-	-	-14%
	Male, F1 (n = 3-5)								
	Mean (SD)	44.8 (4.55)	48.6 (7.6)	46.3 (8.2)	47.2 (3.4)	42.6 (6.6)	45.0 (4.3)	46.6 (5.1)	47.6 (12.4)
	% of control ^a	-	8%	3%	5%	-5%	0%	4%	6%
	Female, F1 (n = 3-5)								
	Mean (SD)	50.6 (16.6)	37.8 (13.4)	38.8 (8.2)	49.6 (11.1)	44.8 (13.5)	59.7 (4.9)	41.4 (12.1)	47.0 (10.8)
	% of control ^a	-	-25%	-23%	-2%	-11%	18%	-18%	-7%
	T3 (nmol/L)								
	Male, F0 (n = 5)^b								
	Mean (SD)	0.9 (0.1)	-	-	-	-	-	-	0.8 (0.1)
	% of control ^a	-	-	-	-	-	-	-	-11%
	Female, F0 (n = 5)^b								
Mean (SD)	0.8 (0.2)	-	-	-	-	-	-	0.9 (0.3)	
% of control ^a	-	-	-	-	-	-	-	12%	
Male, F1 (n = 3-5)									
Mean (SD)	0.9 (0.1)	1.2 (0.2)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	0.9 (0.1)	0.9 (0.1)	1.0 (0.1)	
% of control ^a	-	33%	11%	11%	11%	0%	0%	11%	
Female, F1 (n = 3-5)									
Mean (SD)	1.1 (0.3)	1.2 (0.2)	1.1 (0.2)	1.1 (0.1)	1.2 (0.2)	1.4 (0.1)	1.0 (0.1)	1.0 (0.1)	
% of control ^a	-	9%	0%	0%	9%	27%	-9%	-9%	
	Doses (mg/kg-d)								

Reference and study design	Results								
	0		100		300		1,000		
<p>WIL Research (2001) Rats, Crl:CD(SD)IGS BR Gavage 90-d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown Thyroid hormones (total T3/T4) measured by electro-chemiluminescence immunoassay in adults only Data Quality:^d High (1.0) - Note: thyroid hormone metrics were determined to be low quality due to inadequate reporting of thyroid hormone measurement methods and questionable control data.</p>	TSH (ng/mL)								
	Male (n = 5–10)								
	Mean (SD)	0.46 (0.42)	3.29 (3.86)	2.65 (2.10)	3.88 (2.98)				
	% of control ^a	–	615%	476%	743%				
	Female (n = 5–10)								
	Mean (SD)	0.46 (0.31)	1.42 (1.11)	3.96 (5.15)	2.43 (1.74)				
	% of control ^a	–	209%	761%	428%				
	T4 (µg/dL)								
	Male (n = 9–10)								
	Mean (SD)	7.87 (1.22)	6.34* (1.22)	6.28* (1.03)	4.97* (0.76)				
	% of control ^a	–	–19%	–20%	–37%				
	Female (n = 9–10)								
Mean (SD)	5.43 (0.86)	4.96 (0.62)	4.53* (0.88)	4.31* (0.76)					
% of control ^a	–	–9%	–17%	–21%					
T3 (ng/dL)									
Male (n = 9–10)									
Mean (SD)	64.36 (9.55)	58.78 (13.01)	58.96 (13.17)	64.23 (9.55)					
% of control ^a	–	–9%	–8%	0%					
Female (n = 9–10)									
Mean (SD)	73.4 (14.97)	70.78 (19.18)	67.02 (17.22)	70.31 (16.78)					
% of control ^a	–	–4%	–9%	–4%					
<p>van der Ven et al. (2006) Rats, Wistar Gavage 28-d exposure starting on PNW 11 Thyroid hormones (total T3/T4) were measured by radioimmunoassay Data Quality:^d High (1.3)</p>	Doses (mg/kg-d)								
		0	0.3	1	3	10	30	100	200
	T4 (nmol/L)								
	Male (n = 4–5)								
	Mean (SD)	40.2 (3.6)	40.4 (5.0)	40.6 (5.3)	49.4 (7.2)	43.3 (1.3)	41.9 (4.6)	35.4 (4.2)	41.4 (3.5)
	% of control ^a	–	0%	1%	23%	8%	4%	–12%	3%
	Female (n = 4–5)**								
	Mean (SD)	41.3 (2.6)	41.9 (3.1)	40.2 (7.3)	37.2 (4.7)	38.6 (1.7)	38 (6.1)	35.8 (5.2)	30.4 (5.9)
	% of control ^a	–	1%	–3%	–10%	–7%	–8%	–13%	–26%
	T3 (nmol/L)								
Male (n = 4–5)									
Mean (SD)	0.81 (0.06)	0.84 (0.14)	0.85 (0.16)	0.89 (0.04)	0.97 (0.16)	0.90 (0.13)	0.82 (0.06)	0.89 (0.05)	

Reference and study design	Results								
	% of control ^a	-	4%	5%	10%	20%	11%	1%	10%
	Female (n = 4-5)								
	Mean (SD)	0.91 (0.10)	0.84 (0.15)	0.88 (0.12)	0.81 (0.11)	0.80 (0.09)	0.74 (0.15)	0.92 (0.20)	0.82 (0.13)
	% of control ^a	-	-8%	-3%	-11%	-12%	-19%	1%	-10%
Saegusa et al. (2009) Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11 Thyroid hormones were measured by electrochemiluminescence immunoassay in males only Data Quality: ^d High (1.2)	Doses (mg/kg-d)^c								
		0		15		146		1,505	
	TSH (ng/mL)								
	Male, F1, PND 20 (n = 10)								
	Mean (SD)	5.40 (0.62)		6.66 (1.24)		6.07 (1.41)		7.00* (1.31)	
	% of control ^a	-		23%		12%		30%	
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	4.74 (0.62)		5.81 (1.72)		5.36 (1.11)		4.96 (0.8)	
	% of control ^a	-		23%		13%		5%	
	T4 (µg/dL)								
	Male, F1, PND 20 (n = 10)								
	Mean (SD)	4.39 (0.93)		4.20 (0.77)		4.78 (0.49)		4.20 (0.52)	
	% of control ^a	-		-4%		9%		-4%	
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	4.77 (0.7)		4.84 (0.59)		5.21 (0.65)		5.20 (0.98)	
	% of control ^a	-		1%		9%		9%	
	T3 (ng/mL)								
	Male, F1, PND 20 (n = 10)								
	Mean (SD)	1.09 (0.11)		1.13 (0.12)		1.06 (0.08)		0.93* (0.10)	
	% of control ^a	-		4%		-3%		-15%	
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	0.96 (0.06)		0.93 (0.07)		0.88* (0.05)		0.89* (0.06)	
	% of control ^a	-		-3%		-8%		-7%	
Thyroid histopathology									
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2 offspring: continuous maternal Exposure throughout gestation/lactation	Doses (mg/kg-d)								
	Male, F0	0		10		101		1,008	
	Female, F0	0		14		141		1,363	
	Male, F1	0		11		115		1,142	
	Female, F1	0		14		138		1,363	
	Decreased thyroid follicle size								
	Male, F0 (n = 23-24)								
	Incidence	0/24		0/24		6/24*		20/23*	
	Female, F0 (n = 23-24)								
	Incidence	0/24		0/24		5/24*		11/23*	
	Male, F1 (n = 22-24)								
	Incidence	0/24		0/24		2/22		11/24*	
	Female, F1 (n = 24)								

Reference and study design	Results								
Data Quality: ^d High (1.0)	Incidence	0/24	1/24	5/24*	13/24*				
	Thyroid follicular cell hypertrophy								
	Male, F0 (n = 23–24)								
	Incidence	0/24	0/24	3/24	1/23				
	Female, F0 (n = 23–24)								
	Incidence	0/24	0/24	2/24	0/23				
	Male, F1 (n = 22–24)								
	Incidence	0/24	0/24	0/22	0/24				
WIL Research (2001) Rats, Crl:CD(SD)IGS BR Gavage 90-d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown Data Quality: ^d High (1.0)	Doses (mg/kg-d)								
		0	100	300	1,000				
	Thyroid follicular cell hypertrophy (total incidence, includes all severities)								
	Male (n = 9–10)								
	Incidence	1/10	1/10	5/10	8/9				
	Female (n = 9–10)								
	Incidence	0/10	0/10	4/9	7/10				
	van der Ven et al. (2006) Rats, Wistar Gavage 28-d exposure in adults starting on PNW 11 Data Quality: ^d High (1.3)	Doses (mg/kg-d)							
		0	0.3	1	3	10	30	100	200
Thyroid activation									
Dose-dependent increases in thyroid activation (i.e., follicle size, epithelial cell height, vacuolization, and nuclear size) were reported qualitatively for both males and females.									
WIL Research (1997) Rats, Sprague-Dawley Gavage 28-d exposure starting on ~PNW 6 followed by a 14-d recovery period Data Quality: ^d High (1.3)	Doses (mg/kg-d)								
		0	125	350	1,000				
	Thyroid follicular cell hypertrophy (total incidence, includes all severities)								
	Male (n = 6)								
	Incidence	6/6	6/6	6/6	6/6				
	Female (n = 6)								
	Incidence	6/6	5/6	6/6	6/6				
	Colloid loss (total incidence, includes all severities)								
Male (n = 6)									
Incidence	5/6	4/6	6/6	6/6					
Female (n = 6)									
Incidence	4/6	4/6	6/6	6/6					

Reference and study design	Results				
Saegusa et al. (2009) Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11 Data Quality: ^d High (1.2)	Doses (mg/kg-d)^c				
	0	15	146	1,505	
	Thyroid follicular cell hypertrophy				
	Female, F0 (n = 10)				
	Incidence	3/10	5/10	6/10	9/10*
Males and females, F1: no treatment-related histopathological effects.					
Maranghi et al. (2013) Mice, BALB/c Females only Diet 28-d exposure starting on PND 26 Data Quality: ^d High (1.3)	Doses (mg/kg-d)				
	0			199	
	Female (n = 6–8)				
	Colloid area (µm²)				
	Mean (SD)	1,718 (403)		1,270 (452)	
	% of control ^a	–		–26%	
	Follicle area (µm²)				
	Mean (SD)	2,402 (500)		1,927 (610)	
	% of control ^a	–		–20%	
	Follicle:colloid ratio				
Mean (SD)	1.41 (0.07)		1.53* (0.07)		
% of control ^a	–		9%		
<i>Thyroid weight</i>					
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Thyroid weight measured in adults only Data Quality: ^d High (1.0)	Doses (mg/kg-d)				
	Male, F0	0	10	101	1,008
	Female, F0	0	14	141	1,363
	Male, F1	0	11	115	1,142
	Female, F1	0	14	138	1,363
	Relative thyroid weight (mg/100 g BW)				
	Male, F0 (n = 22–24)				
	Mean (SD)	4.28 (0.71)	4.17 (0.77)	4.09 (0.73)	5.17* (1.00)
	% of control ^a	–	–3%	–4%	21%
	Female, F0 (n = 17–24)				
	Mean (SD)	6.38 (0.89)	5.99 (1.27)	6.47 (1.32)	7.20 (1.30)
	% of control ^a	–	–6%	1%	13%
	Male, F1 (n = 22–24)				
	Mean (SD)	4.03 (0.79)	4.22 (0.63)	4.15 (0.72)	4.96* (0.87)
% of control ^a	–	5%	3%	23%	
Female, F1 (n = 13–22)					
Mean (SD)	6.01 (1.01)	6.08 (1.05)	6.54 (1.36)	7.76* (1.36)	
% of control ^a	–	1%	9%	29%	
Doses (mg/kg-d)					

Reference and study design	Results															
	0	0.1	0.3	1	3	10	30	100								
van der Ven et al. (2009) Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Data Quality: ^d High (1.2)	Absolute thyroid weight (mg)															
	Male, F1 (n = 5)															
	Mean (SD)	26 (3)	24 (3)	30 (5)	26 (3)	26 (3)	25 (5)	25 (5)	26 (1)							
	% of control ^a	–	–8%	15%	0%	0%	–4%	–4%	0%							
Female, F1 (n = 5)																
Mean (SD)	24 (5)	21 (3)	19 (4)	20 (5)	22 (4)	20 (4)	19 (6)	22 (3)								
% of control ^a	–	–12%	–21%	–17%	–8%	–17%	–21%	–8%								
WIL Research (2001) Rats, CrI:CD(SD)IGS BR Gavage 90-d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown Data Quality: ^d High (1.0)	Doses (mg/kg-d)															
	0		100		300		1,000									
	Relative thyroid weight (mg/100 mg BW)															
	Male (n = 9–10)															
Mean (SD)	5 (1.2)		5 (1.6)		5 (1.6)		5 (1.3)									
% of control ^a	–		0%		0%		0%									
Female (n = 10)																
Mean (SD)	6 (1.2)		7 (1.8)		6 (1.2)		7 (1.4)									
% of control ^a	–		17%		0%		17%									
van der Ven et al. (2006) Rats, Wistar Gavage 28-d exposure starting on PNW 11 Data Quality: ^d High (1.3)	Doses (mg/kg-d)															
	0		0.3		1		3		10		30		100		200	
	Relative thyroid weight (g/g BW × 100,000)															
	Male (n = 3–5)															
Response	7.33 (1.03)	4.08 (0.36)	6.13 (1.68)	6.97 (0.10)	6.02 (2.09)	6.28 (0.53)	5.54 (0.39)	6.46 (1.14)								
% of control ^a	–	–44%	–16%	–5%	–18%	–14%	–24%	–12%								
Female (n = 4–5)**																
Response	5.98 (0.60)	6.62 (0.68)	8.98 (1.03)	5.26 (1.35)	7.13 (0.60)	9.52 (0.59)	9.41 (2.26)	9.59 (0.88)								
% of control ^a	–	11%	50%	–12%	19%	59%	57%	60%								
Saegusa et al. (2009) Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-	Doses (mg/kg-d)^c															
	0		14.8		146.3		1,505									
	Relative thyroid weight (mg/100 g BW)															
	Female, F0 (n = 10)															
Mean (SD)	5.73 (0.90)		6.75 (0.99)		6.30 (0.80)		7.47* (1.05)									
% of control ^a	–		18%		10%		30%									

Reference and study design	Results				
exposure period through PNW 11 Data Quality: ^d High (1.2)	Male, F1, PNW 11 (n = 10)				
	Mean (SD)	4.85 (0.69)	5.66 (0.67)	5.78* (0.82)	6.20* (1.03)
	% of control ^a	–	17%	19%	28%
	Female, F1, PNW 11 (n = 10)				
	Mean (SD)	8.20 (2.94)	6.84 (0.81)	7.35 (0.87)	7.72 (0.83)
	% of control ^a	–	–17%	–10%	–6%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.

**Significant dose response trend as reported by study authors.

^aPercent change compared to control calculated as: (treated value – control value)/control value × 100.

^bNot measured; only control and high-dose values reported for endocrine parameters in the F0 animals.

^cTime-weighted averages (TWAs) for each exposure group were calculated by multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PNDs 1–9, and PNDs 9–20 by the number of inclusive days of exposure for each time.

^dBased on OPPT data evaluation criteria

BW = body weight; GD = gestation day; PNW = postnatal week

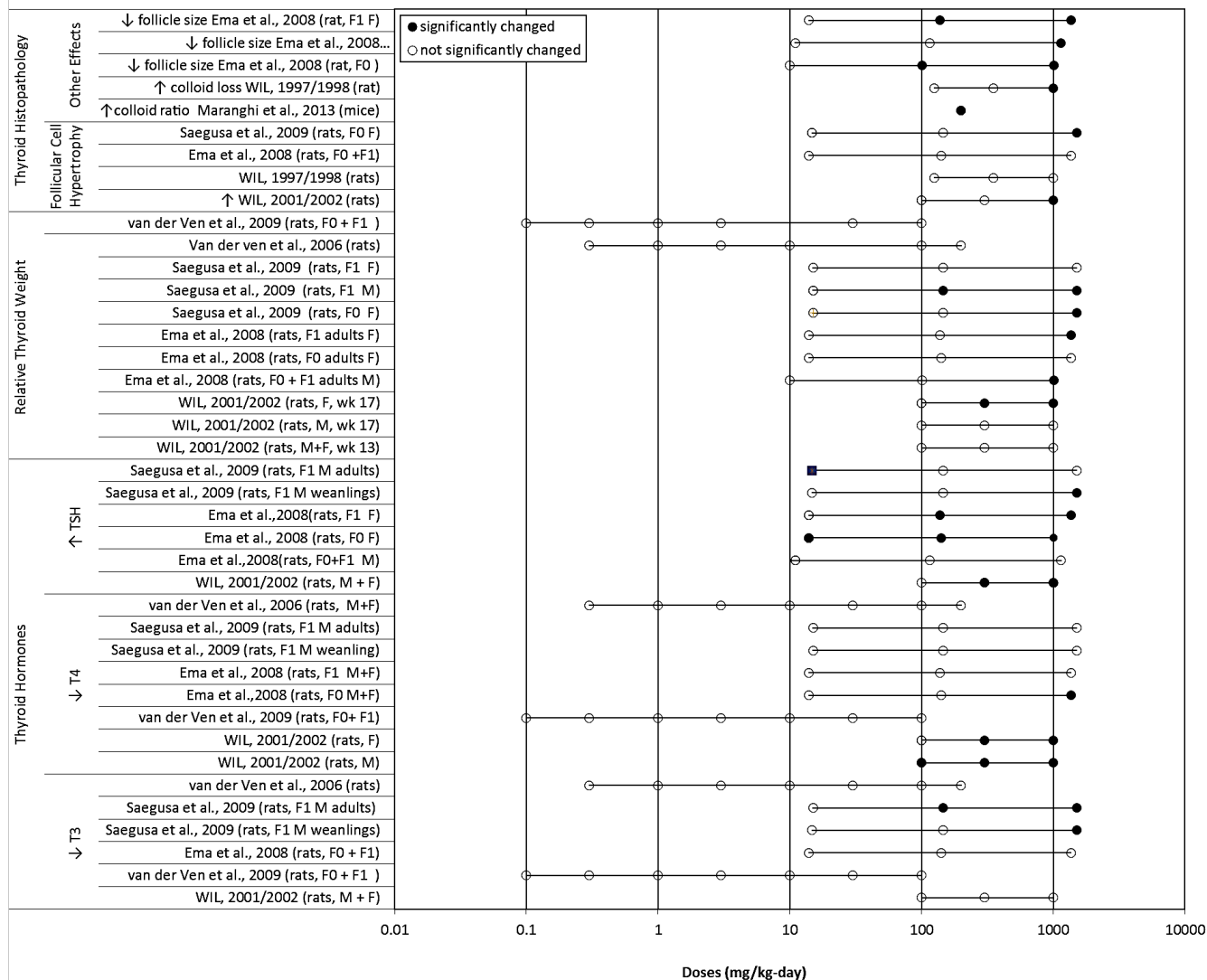


Figure 1-1. Exposure response array of thyroid effects following oral exposure. All studies scored a High in data quality evaluation.

1.1.6 Mechanistic Evidence

Available mechanistic data suggest that HBCD may interfere with normal thyroid hormone function. Indirectly, HBCD may decrease circulating thyroid hormone levels by inducing liver xenobiotic enzymes that are responsible for metabolizing thyroid hormones. Directly, HBCD may act via the thyroid receptor and regulate thyroid-responsive genes. Evidence to support these hypothesized modes of action (MOAs) are reviewed below. Other related, but less supported possible mechanisms, such as competition for thyroid hormone binding proteins and dysregulation of deiodinases, are also included in this review. The complex interplay of physiologic processes that regulate thyroid hormone homeostasis and possible sites of disruption by HBCD are summarized in Figure 1-2 and the text below.

1.1.6.1 Indirect Pathway: Increased Clearance of Thyroid Hormones

Results from short-term in vivo studies suggest that HBCD induces uridine diphosphate glucuronyl transferase (UGT), an enzyme that regulates metabolism and irreversible elimination of T4 ([Shelby et al., 2003](#); [Vansell and Klaassen, 2002](#); [Kelly, 2000](#)). HBCD-mediated activation of UGT has been observed in both rodent and non-mammalian models ([Crump et al., 2010](#); [Cantón et al., 2008](#); [Crump et al., 2008](#); [Palace et al., 2008](#); [van der Ven et al., 2006](#)). In rats, UGT activity showed dose-related increases in both males and females exposed to up to 200 mg/kg-day ([van der Ven et al., 2006](#)) and gene transcription in males exposed to 30 and 100 mg/kg-day HBCD ([Cantón et al., 2008](#)). Additional support for this mechanism is provided by data obtained from fish and avian models. Activity of liver UGT increased by approximately 45% in juvenile rainbow trout exposed to α - or β -HBCD isomers in the diet for 56 days ([Palace et al., 2008](#)). Similarly, the technical mixture or α -HBCD induced hepatic expression of a UGT1A1 ortholog in chicken embryos ([Crump et al., 2010](#); [Crump et al., 2008](#)). These data suggest that HBCD-mediated induction of UGT could lower serum thyroid hormone levels through increased thyroid hormone catabolism and excretion ([Kato et al., 2008](#); [Klaassen and Hood, 2001](#)). As shown in Figure 1-2, decreased levels of circulating thyroid hormones trigger activation of HPT axis feedback mechanisms, which stimulate the release of TSH.

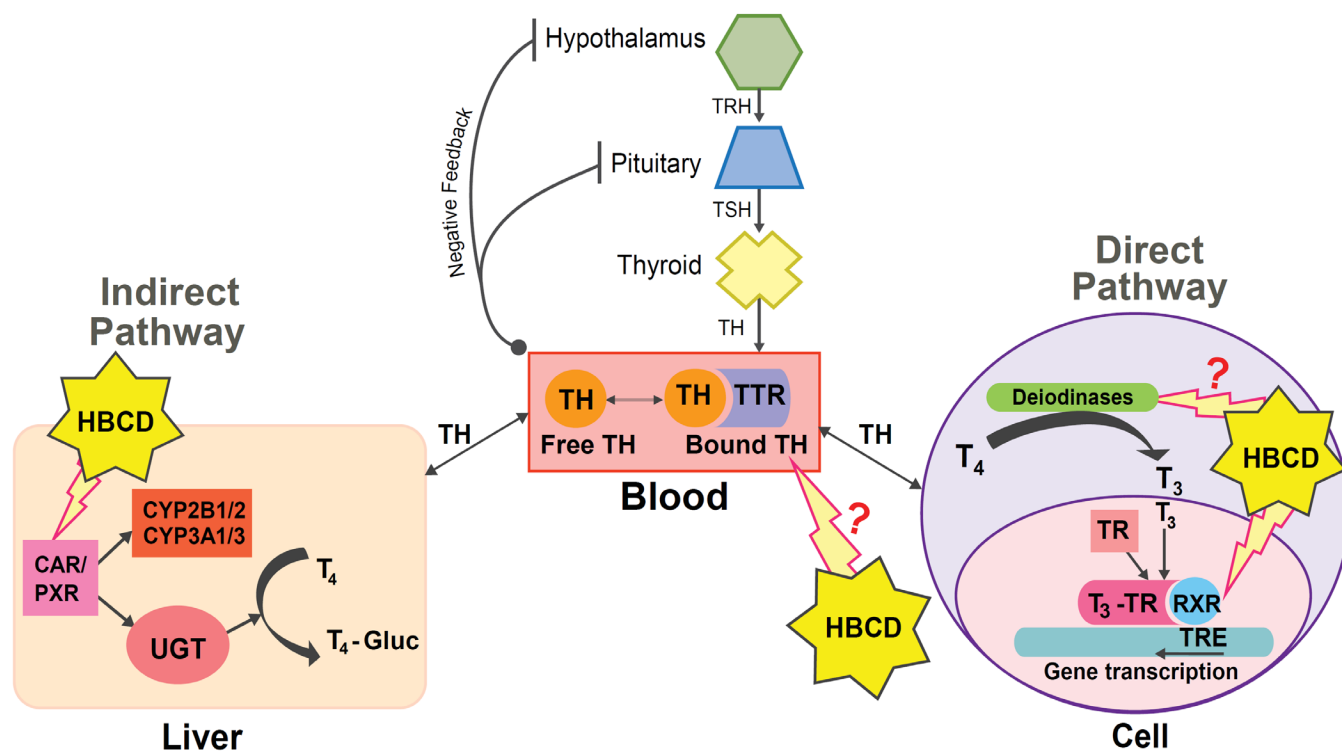
Although the exact mechanism by which HBCD induces UGT is unclear, there is some evidence to indicate that this effect may be mediated by interaction with the constitutive androstane receptor (CAR) and/or pregnane X receptor (PXR). Often referred to as xenobiotic sensors, these nuclear receptors bind to numerous exogenous compounds and regulate metabolizing enzymes ([Chen et al., 2003](#); [Mackenzie et al., 2003](#)). HBCD activated CAR in a human breast cancer cell line ([Sakai et al., 2009](#)). Although [Sakai et al. \(2009\)](#) is the only study that directly investigated interaction of HBCD with CAR/PXR, these results are supported by studies in HBCD-exposed animal models showing activation of several other enzymes that are regulated by these nuclear receptors ([Omiecinski et al., 2011](#); [Rosenfeld et al., 2003](#); [Ueda et al., 2002](#)). Upregulation or increased activity of CYP2B1/2 and CYP3A1/3 was reported in HBCD-exposed rats ([Cantón et al., 2008](#); [Germer et al., 2006](#)) and chicken embryos ([Crump et al., 2010](#); [Crump et al., 2008](#)). Pentoxoresorufin-O-depethylase activity, a biomarker of CYP2B1, was also increased in HBCD-exposed fish ([Zhang et al., 2008](#)). Additionally, liver weight increases in rats and mice are often associated with hepatic microsomal induction ([Amacher et al., 1998](#)); thus, the HBCD-induced liver weight increases (16–108%) observed in rodents ([Maranghi et al., 2013](#); [Saegusa et al., 2009](#); [WIL Research, 2001](#)) are consistent with the findings from these mechanistic studies. Taken together, these data support the hypothesis that perturbation of thyroid hormones following HBCD exposure is driven by indirect induction of UGT through interaction with CAR/PXR.

1.1.6.2 Direct Pathway: Stimulation of Thyroid Hormone Receptor (TR) Signaling at the Cellular Level

Thyroid hormones bind with the thyroid receptor (TR) to form the thyroid hormone/TR complex. When formed, this complex translocates into the nucleus to activate transcription via the thyroid hormone response element (TRE). Xenobiotic chemicals can alter TRE transcription by interfering with the formation of the thyroid hormone/TR complex or its ability to interact with the TRE ([Kitamura et al., 2005](#)). Although it is unclear whether HBCD binds to the TR, there is evidence to support treatment-related TR activation (e.g., proliferation, gene expression).

Several in vitro models indicate that HBCD may act as a TR agonist. Two studies evaluated the effect of HBCD on rat pituitary tumor cells (GH3 cells) that proliferate via TR activation by T3. Both reported that the technical mixture of HBCD increased GH3 cell proliferation in the presence of T3 ([Hamers et al., 2006](#); [Schriks et al., 2006a](#)). In the absence of T3, α -HBCD, but not other isomers, still induced proliferation; however, the magnitude of the effect was small ([Hamers et al., 2006](#)). Maximal proliferation stimulation by HBCD was observed when T3 was added simultaneously, which mimics in vivo conditions.

Interaction of HBCD with the TR was also examined in a *Xenopus laevis* tadpole tail tip regression model that simulates amphibian metamorphosis. In organ culture, the tail tissue responds to T3 by undergoing TR-mediated regression ([Furlow et al., 2004](#); [Shaffer, 1963](#)). [Schriks et al. \(2006b\)](#) demonstrated that the T3-induced tadpole tail tip regression was potentiated by the technical mixture of HBCD. In HeLa cells that constitutively overexpress TR α and were transfected with TRE luciferase construct, HBCD increased TRE transcription by about 1.8-fold ([Yamada-Okabe et al., 2005](#)). Two studies using green monkey kidney fibroblast (CV-1) cells transfected with *Xenopus* TR/TRE luciferase constructs provide inconsistent results regarding the effects of HBCD on TR activation ([Ibhazehiebo et al., 2011a](#); [Schriks et al., 2007](#)). Notably, this model has less biological relevance in studying TR activation when compared to those that endogenously express the TR (e.g., “T-screen” assay, *X. laevis* tadpole tail tip regression, and HeLa cells).



Indirect Pathway. HBCD induces UGT in the liver, increasing TH elimination, lowering circulating TH levels and activating the hypothalamic-pituitary-thyroid feedback axis. Direct Pathway: HBCD may interfere with TR signaling by interfering with binding to the TRE. Other: HBCD may alter thyroid homeostasis through competitive binding with TTR or dysregulation of deiodinases. CAR/PXR = constitutive antrostande receptor/pregnane X receptor; Gluc = glucuronide; RXR = retinoid X receptor; T₄ = Thyroxine; T₃ = triiodothyronine; TH = thyroid hormone; TR = thyroid receptor; TRE = thyroid hormone response element; TRH = thyrotropin-releasing hormone; TSH = thyroid stimulating hormone; TTR = transthyretin; UGT = uridine diphosphate glucuronyltransferase;

Figure 1-2. Hypothesized MOAs for thyroid effects of HBCD (adapted from [Miller et al. \(2009\)](#))

1.1.6.3 Other Mechanistic Information

Environmental chemicals can alter circulating levels of free T₃ and T₄ by competitively binding with the serum transport protein, transthyretin (TTR) ([Schussler, 2000](#); [Lans et al., 1993](#)) or interacting with deiodinase enzymes ([Klammer et al., 2007](#); [Morse et al., 1993](#)). Two in vitro studies provide limited evidence of HBCD interaction with TTR. [Crump et al. \(2008\)](#) reported a >2-fold inhibition of TTR messenger ribonucleic acid (mRNA) transcription in chicken embryonic hepatocytes following exposure to both the technical mixture and α -HBCD for 24 hours, but this effect diminished after treatment for 36 hours. In a TTR replacement assay, α - and β -HBCD showed low potency (IC₅₀ > 10 μ M), whereas the technical mixture and γ -isomer showed no ability to compete with T₄ binding sites ([Hamers et al., 2006](#)). Additionally, dysregulation of deiodinase enzymes that catalyze the deiodination of T₄ to T₃ can disrupt thyroid hormone metabolism ([Klammer et al., 2007](#); [Morse et al., 1993](#)). In the liver, total T₄ to T₃ conversion was decreased by approximately 40% in juvenile rainbow trout fed α -, β -, or γ -isomers for 56 days ([Palace et al., 2008](#)); however, the same research group later reported that β - and γ -HBCD increased conversion by approximately 60% in the same species after a 32-day

dietary exposure ([Palace et al., 2010](#)). Differences in the way enzyme activity was measured in the two experiments may have contributed to the disparate outcomes. Overall, these data provide limited evidence for a role of HBCD in dysregulating the conversion of T4 to T3 in the liver.

1.2 Liver Effects

1.2.1 Human Evidence

The potential for HBCD to affect the liver has not been investigated in humans.

1.2.2 Animal Evidence

Several rodent studies have evaluated hepatic effects, including changes in liver weight, liver chemistry, and histopathology, following oral exposure to HBCD. A summary of liver effects associated with HBCD exposure is presented in Table 1-3 and Figure 1-3. Effect categories with stronger evidence are presented first, with individual studies ordered by study duration and then species. If not otherwise indicated, endpoint measurements were made in adults.

1.2.2.1 Liver Weight

Effects on liver weight were evaluated in eight studies in rats ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#); [van der Ven et al., 2006](#); [WIL Research, 2001, 1997](#)) and mice ([Yanagisawa et al., 2014](#); [Maranghi et al., 2013](#)). With the exception of three studies that presented only absolute liver weight ([Yanagisawa et al., 2014](#); [van der Ven et al., 2009](#); [van der Ven et al., 2006](#)), study authors reported both absolute and relative liver weights. This discussion focuses on relative liver weight changes, as this measure has been shown in the general literature to be more informative in evaluating liver toxicity when there are changes in body weight ([Bailey et al., 2004](#)); absolute weight data were considered when relative weights were not available.

Statistically significant increases in relative liver weight were reported in five studies in rats ([Saegusa et al., 2009](#); [Ema et al., 2008](#); [WIL Research, 2001, 1997](#)) and mice ([Maranghi et al., 2013](#)) that utilized similar dose ranges (10–1,505 mg/kg-day), generally at concentrations ≥ 100 mg/kg-day.

Study authors reported a significant positive trend with dose for absolute liver weight in adult female, but not male, rats exposed to HBCD for 28 days ([van der Ven et al., 2006](#)), but a later study by the same research group did not see a similar effect in F1 rats from a one-generation study ([van der Ven et al., 2009](#)). In a study designed to investigate the influence of HBCD exposure on metabolic function ([Yanagisawa et al., 2014](#)), absolute liver weight was examined in male mice dosed once per week for 105 days while being fed either a standard diet or a high-fat diet (created by mixing lard into the feed) at HBCD dose levels (0.002–0.7 mg/kg-week) several orders of magnitude lower than other studies. Changes in absolute liver weight were not observed in mice receiving the standard diet but mice receiving the high-fat diet showed treatment-related increases. The increased absolute liver weight corresponded with significant increases in body weight in these animals.

In three rat studies that evaluated animals 2–8 weeks after the end of exposure, liver weight returned to control levels in all dose groups ([Saegusa et al., 2009](#); [WIL Research, 2001, 1997](#)).

1.2.2.2 Liver Histopathology

Histopathological changes were investigated following oral exposure to HBCD in six studies in rats ([Saegusa et al., 2009](#); [Ema et al., 2008](#); [WIL Research, 2001, 1997](#)) and mice ([Yanagisawa et al., 2014](#); [Maranghi et al., 2013](#)). Increased hepatocellular vacuolation, which can reflect a normal physiological process as well as a response to a toxic agent ([Henics and Wheatley, 1999](#)), was the most consistently observed histopathological change, with effects seen in male and female rats and female mice following multiple exposure durations at doses ranging from 100 to 1,505 mg/kg-day ([Maranghi et al., 2013](#); [Saegusa et al., 2009](#); [WIL Research, 2001, 1997](#)). One of these studies stained liver sections with lipid- and glycogen-specific stains (Oil Red O and periodic acid Schiff's reagent, respectively) and characterized the vacuoles as lipid filled ([WIL Research, 2001](#)). With the exception of hypertrophy, which was increased in high-dose females in the study by [WIL Research \(2001\)](#), no other significant histopathological changes were reported in the available rat studies; however, some histopathologic changes were observed in mouse studies. Low HBCD exposures (up to 0.7 mg/kg-week) in male mice showed no histological changes in mice fed a standard diet; however, increases in microvesicular fatty changes (steatosis) and hypertrophy (characterized as hepatocyte ballooning) were observed in the high-dose group given a high-fat diet relative to the high-fat controls. Confidence in these findings is reduced because other dose groups were not evaluated histologically and data were presented qualitatively only ([Yanagisawa et al., 2014](#)). In a second mouse study, statistically significant increases in the incidence of lymphocytic infiltration and tissue congestion, indicators of inflammation, were observed in female mice administered 199 mg/kg-day ([Maranghi et al., 2013](#)).

In two rat studies that evaluated animals 2–4 weeks after the end of exposure, histopathological changes returned to control levels in all dose groups ([WIL Research, 2001, 1997](#)).

1.2.2.3 Liver Chemistry

Changes in serum liver enzyme levels were investigated as potential indicators of liver damage following short-term and subchronic oral exposure to HBCD in five studies in rats ([van der Ven et al., 2009](#); [van der Ven et al., 2006](#); [WIL Research, 2001, 1997](#)) and mice ([Yanagisawa et al., 2014](#)).

Measures of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicators of hepatocellular injury, showed no biologically or statistically significant increases with HBCD exposure; indeed, animals in the high-dose groups often showed decreases in these enzyme levels ([Yanagisawa et al., 2014](#); [van der Ven et al., 2009](#); [van der Ven et al., 2006](#); [WIL Research, 2001, 1997](#)). Although it is generally accepted that increases in serum ALT greater than 100% of controls is suggestive of hepatocellular damage ([Ema, 2008](#); [Boone et al., 2005](#)), the biological significance of decreased aminotransferase levels is unclear.

Serum γ -glutamyltransferase (GGT) and serum alkaline phosphatase (ALP) activities, markers of hepatobiliary injury, were also reported in four studies ([van der Ven et al., 2009](#); [van der Ven et al., 2006](#); [WIL Research, 2001, 1997](#)). GGT was significantly increased in male and female rats exposed to 1,000 mg/kg-day for 90 days; this effect was not observed following a 4-week recovery period ([WIL Research, 2001](#)) or a shorter (28-day) exposure ([WIL Research, 1997](#)). In general, ALP activity was consistently decreased, sometimes statistically significantly, in male and female rats ([van der Ven et al., 2009](#); [van der Ven et al., 2006](#); [WIL Research, 2001, 1997](#)).

Although decreased ALP levels are not generally associated with liver injury, they can be a marker of vitamin B₆ (pyridoxal phosphate) or zinc deficiency ([Hall et al., 2012](#); [Waner and Nyska, 1991](#)).

Table 1-3. Evidence pertaining to liver effects in animals following exposure to HBCD

Reference and study design	Results								
<i>Liver weight</i>									
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Data Quality: ^c High (1.0)	Doses (mg/kg-d)								
	Male, F0	0	10	101	1,008				
	Female, F0	0	14	141	1,363				
	F1 offspring^a	0	17	168	1,570				
	Male, F1	0	11	115	1,142				
	Female, F1	0	14	138	1,363				
	F2 offspring^a	0	15	139	1,360				
	Relative liver weight (g/100 g BW)								
	Male, F0 (n = 22–24)								
	Mean (SD)	3.23 (0.26)	3.33 (0.24)	3.41* (0.31)	4.06* (0.22)				
	% of control ^b	–	3%	6%	26%				
	Female, F0 (n = 17–24)								
	Mean (SD)	4.69 (0.52)	4.76 (0.65)	4.88 (0.48)	6.07* (0.47)				
	% of control ^b	–	1%	4%	29%				
	Male, F1, PND 26 (n = 17–23)								
	Mean (SD)	4.60 (0.37)	4.60 (0.32)	5.05* (0.32)	6.00* (0.44)				
	% of control ^b	–	0%	10%	30%				
Female, F1, PND 26 (n = 14–23)									
Mean (SD)	4.57 (0.35)	4.59 (0.28)	5.02* (0.32)	6.07* (0.36)					
% of control ^b	–	0%	10%	33%					
Male, F1, adult (n = 22–24)									
Mean (SD)	3.27 (0.18)	3.34 (0.26)	3.37 (0.25)	3.86* (0.28)					
% of control ^b	–	2%	3%	18%					
Female, F1, adult (n = 13–22)									
Mean (SD)	4.18 (0.42)	4.39 (0.44)	4.38 (0.47)	5.05* (0.50)					
% of control ^b	–	5%	5%	21%					
Male, F2, PND 26 (n = 13–22)									
Mean (SD)	4.72 (0.59)	4.74 (0.35)	5.04* (0.4)	6.00* (0.25)					
% of control ^b	–	0%	7%	27%					
Female, F2, PND 26 (n = 13–22)									
Mean (SD)	4.70 (0.27)	4.70 (0.28)	4.94 (0.32)	5.89* (0.44)					
% of control ^b	–	0%	5%	25%					
van der Ven et al. (2009) Rats, Wistar Diet	Doses (mg/kg-d)								
	0	0.1	0.3	1	3	10	30	100	
	Absolute liver weight (g)								
Male, F1, PNW 11 (n = 4–5)									

Reference and study design	Results									
<p>One generation</p> <p>F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating</p> <p>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11</p> <p>Data Quality:^c High (1.2)</p>	Mean (SD)	11.9 (1.5)	12.3 (0.4)	12.7 (0.8)	14.4 (2.0)	12.2 (1.7)	12.1 (0.8)	14.0 (2.8)	12.0 (0.5)	
	% of control ^b	–	3%	7%	21%	3%	2%	18%	1%	
	Female, F1, PNW 11 (n = 4–5)									
	Mean (SD)	7.7 (0.9)	7.9 (0.8)	7.8 (1.4)	8.3 (0.5)	7.7 (0.8)	8.3 (0.5)	9.0 (1.1)	8.4 (0.6)	
% of control ^b	–	3%	1%	8%	0%	8%	17%	9%		
<p>WIL Research (2001)</p> <p>Rats, Crl:CD(SD)IGS BR</p> <p>Gavage</p> <p>90-d exposure starting on ~PNW 7 followed by a 28-d recovery period</p> <p>Recovery data not shown</p> <p>Data Quality:^c High (1.0)</p>	Doses (mg/kg-d)									
	0		100		300		1,000			
	Relative liver weight (g/100 g BW)									
	Male (n = 10)									
Mean (SD)	2.71 (0.12)		3.18* (0.23)		3.13* (0.27)		3.86* (0.16)			
% of control ^b	–		17%		17%		42%			
Female (n = 10)										
Mean (SD)	2.89 (0.21)		3.58* (0.27)		3.58* (0.35)		4.31* (0.29)			
% of control ^b	–		24%		24%		49%			
<p>van der Ven et al. (2006)</p> <p>Rats, Wistar</p> <p>Gavage</p> <p>28-d exposure starting on PNW 11</p> <p>Data Quality:^c High (1.3)</p>	Doses (mg/kg-d)									
	0	0.3	1	3	10	30	100	200		
	Absolute liver weight (g)									
	Male (n = 4–5)									
Mean (SD)	13.9 (0.7)	17.1 (3.4)	16.2 (3.0)	15.0 (1.6)	17.7 (2.3)	15.7 (0.5)	16.4 (2.3)	16.4 (3.2)		
% of control ^b	–	23%	17%	8%	27%	13%	18%	18%		
Female (n = 4–5)**										
Mean (SD)	9.7 (1.0)	8.9 (1.1)	8.6 (1.3)	9.5 (0.4)	8.9 (0.6)	11.0 (1.0)	13.0 (0.5)	11.6 (0.6)		
% of control ^b	–	–8%	–11%	–2%	–8%	13%	34%	20%		
<p>WIL Research (1997)</p> <p>Rats, Sprague-Dawley</p> <p>Gavage</p>	Doses (mg/kg-d)									
	0		125		350		1,000			
	Relative liver weight (g/100 g BW)									
	Male (n = 6)									
Mean (SD)	3.68 (0.16)		4.05 (0.24)		4.29* (0.29)		4.76* (0.44)			
% of control ^b	–		10%		17%		29%			

Reference and study design	Results				
28-d exposure starting on ~PNW 6 followed by a 14-d recovery period Recovery data not shown Data Quality: ^c High (1.3)	Female (n = 6)				
	Mean (SD)	3.84 (0.39)	4.47* (0.26)	4.69* (0.59)	5.30* (0.25)
	% of control ^b	–	16%	22%	38%
Saegusa et al. (2009) Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11 Data Quality: ^c High (1.2)	Doses (mg/kg-d)^c				
		0	15	146	1,505
	Relative liver weight (g/100 g BW)				
	Male, F1, PND 20 (n = 10)				
	Mean (SD)	3.68 (0.11)	3.82 (0.31)	3.98 (0.15)	4.66* (0.35)
	% of control ^b	–	4%	8%	27%
	Female, F1, PND 20 (n = 10)				
	Mean (SD)	3.77 (0.17)	3.83 (0.23)	4.01 (0.25)	4.83* (0.26)
	% of control ^b	–	2%	6%	28%
	Male, F1, PNW 11 (n = 10)				
Mean (SD)	3.45 (0.27)	3.81* (0.23)	3.58 (0.24)	3.53 (0.22)	
% of control ^b	–	10%	4%	2%	
Female, F1, PNW 11 (n = 10)					
Mean (SD)	3.35 (0.20)	3.59 (0.19)	3.44 (0.25)	3.30 (0.22)	
% of control ^b	–	7%	3%	–1%	
Yanagisawa et al. (2014) Mice, C57BL/6 Males only Gavage Animals dosed once weekly 15-week exposure starting on PNW 6 Dose groups split between standard and high-fat diets Data Quality: ^c Unacceptable (4)*	Doses (mg/kg-wk)				
		0	0.00175	0.035	0.7
	Absolute liver weight (mg), standard diet				
	Male (n = 6)				
	Mean (SE)	1,261 (54.8)	1,283 (36.8)	1,159 (21.9)	1,165 (49.4)
	% of control ^b	–	2%	–8%	–8%
Absolute liver weight (mg), high-fat diet					
Male (n = 6)					
Mean (SE)	1,405 (96.4)	1,622 (164)	1,662* (87.9)	1,790* (153)	
% of control ^b	–	15%	18%	27%	
Maranghi et al. (2013) Mice, BALB/c Females only Diet 28-d exposure starting on PND 26 Data Quality: ^c High (1.3)	Doses (mg/kg-d)				
		0		199	
	Relative liver weight (%)				
	Female (n = 10–15)				
Mean (SD)		4.38 (0.49)		5.67* (0.4)	
% of control ^b		–		29%	

Reference and study design	Results				
<i>Liver histopathology</i>					
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2: continuous maternal exposure throughout gestation/lactation Data Quality: ^c High (1.3)	Doses (mg/kg-d)				
	Male, F0	0	10	101	1,008
	Female, F0	0	14	141	1,363
	F1 offspring^a	0	17	168	1,570
	Male, F1	0	11	115	1,142
	Female, F1	0	14	138	1,363
	F2 offspring^a	0	15	139	1,360
	Histopathological findings				
	Histopathological evaluation did not observe any significant effects with HBCD exposure.				
	Data Quality: ^c High (1.3)				
WIL Research (2001) Rats, Crl:CD(SD)IGS BR Gavage 90-d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown Data Quality: ^c High (1.0)	Doses (mg/kg-d)				
	0	100	300	1,000	
	Hepatocellular hypertrophy				
	Male (n = 10)				
	Incidence	0/10	0/10	0/10	0/10
	Female (n = 10)				
	Incidence	0/10	0/10	0/10	5/10
	Hepatocellular vacuolation				
	Male (n = 9–10)				
	Incidence	2/10	6/10	5/10	6/9
Female (n = 10)					
Incidence	3/10	6/10	5/10	9/10	
Other histopathological findings					
Inflammation was also observed in animals from every treatment group with no pattern related to dose.					
Data Quality: ^c High (1.0)					
WIL Research (1997) Rats, Sprague-Dawley Gavage 28-d exposure starting on ~PNW 6 followed by a 14-d recovery period Recovery data not shown Data Quality: ^c High (1.3)	Doses (mg/kg-d)				
	0	125	350	1,000	
	Hepatocellular vacuolation				
	Male (n = 6)				
	Incidence	0/6	0/6	0/6	0/6
	Female (n = 6)				
	Incidence	1/6	4/6	2/6	5/6
	Other histopathological findings				
	Inflammation was also observed in animals from every treatment group with no pattern related to dose.				
	Data Quality: ^c High (1.3)				
Saegusa et al. (2009) Crj:CD(SD)IGS, rat Diet	Doses (mg/kg-d)^c				
	0	15	146	1,505	
Hepatocellular vacuolar degeneration					

Reference and study design	Results									
F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11 Data Quality: ^c High (1.2)	Male, F1, PND 20 (n = 10)									
	Incidence	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	6/10*
	Female, F1, PND 20 (n = 10)									
	Incidence	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	6/10*
Yanagisawa et al. (2014) Mice, C57BL/6 Males only Gavage Animals dosed once weekly 15-wk exposure starting on PNW 6 Dose groups split between standard and high-fat diets Data Quality: ^c Unacceptable (4)*	Doses (mg/kg-wk)									
		0	0.00175	0.035	0.1	0.35	1.05	3.15	9.45	0.7
	Hepatocyte ballooning									
	The study authors observed development of hepatocyte ballooning following oral high-dose exposure in male mice fed a high-fat diet.									
	Microvesicular fatty changes									
	The study authors observed development of severe microvesicular fatty changes following oral high-dose exposure in male mice fed a high-fat diet.									
Treatment-related effects were not observed in mice fed a standard diet.										
Maranghi et al. (2013) BALB/c, mice Females only Diet 28-d exposure starting on PND 26 Data Quality: ^c High (1.3)	Doses (mg/kg-d)									
		0	199							
	Periportal lymphatic filtration									
	Incidence	0/10	6/8*							
	Tissue congestion									
	Incidence	0/10	6/8*							
Vacuolation in hepatocytes										
Incidence	0/10	5/8*								
<i>Liver chemistry</i>										
van der Ven et al. (2009) Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation;	Doses (mg/kg-d)									
		0	0.1	0.3	1	3	10	30	100	
	ALT (U/L)									
	Male (n = 4–5)									
	Mean	37.3	33.6	43.6	43.1	43.3	40.3	38.2	37.2	
	(SD)	(1.8)	(4.7)	(7.8)	(4.2)	(4.4)	(6.8)	(4.7)	(2.6)	
	% of control ^b	–	–10%	17%	16%	16%	8%	2.4%	0%	
	Female (n = 5)									
	Mean	34.7	37.5	39.7	37.3	33.5	30.7	33.9	34.0	
	(SD)	(3.3)	(6.5)	(12.6)	(4.8)	(6.2)	(6.2)	(10.4)	(4.6)	
% of control ^b	–	8%	14%	7%	–3%	–12%	–2%	–2%		

Reference and study design	Results									
dietary exposure post weaning through PNW 11 Data Quality: ^c High (1.2)	ALP (U/L)									
	Male (n = 4–5)									
	Mean	3.22	4.40	3.28	4.80	3.38	3.20	4.60	3.76	
	(SD)	(2.24)	(2.31)	(1.76)	(2.79)	(1.90)	(0.85)	(2.43)	(1.90)	
	% of control ^b	–	37%	2%	49%	5%	–1%	43%	17%	
Female (n = 5)**										
Mean	3.78	2.70	3.82	2.64	1.14	3.82	2.66	1.28		
(SD)	(1.97)	(2.37)	(3.23)	(0.95)	(0.53)	(1.64)	(1.55)	(0.59)		
% of control ^b	–	–29%	1%	–30%	–70%	1%	–30%	–66%		
WIL Research (2001) Rats, Crl:CD(SD)IGS BR Gavage 90-d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown Data Quality: ^c High (1.0)	Doses (mg/kg-d)									
	0		100		300		1,000			
	ALT (U/L)									
	Male (n = 9–10)									
	Mean (SD)	40 (12.8)		31 (4.8)		40 (12)		33 (6)		
	% of control ^b	–		–22%		0%		–18%		
	Female (n = 10)									
	Mean (SD)	28 (4.9)		30 (5.5)		31 (11.7)		35 (10.2)		
	% of control ^b	–		7%		11%		25%		
	ALP (U/L)									
	Male (n = 10)									
	Mean (SD)	103 (21.5)		87 (11.3)		97 (20.1)		87 (17.6)		
	% of control ^b	–		–16%		–6%		–16%		
	Female (n = 10)									
	Mean (SD)	58 (19.4)		38* (10.7)		39* (10.7)		34* (11.1)		
% of control ^b	–		–34%		–33%		–41%			
AST (U/L)										
Male (n = 9–10)										
Mean (SD)	89 (21.9)		74 (16.4)		75 (16.9)		67 (10.9)			
% of control ^b	–		–17%		–16%		–25%			
Female (n = 10)										
Mean (SD)	83 (17.6)		86 (25.5)		72 (19.1)		77 (30.8)			
% of control ^b	–		4%		–13%		–7%			
GGT (U/L)										
Male (n = 9–10)										
Mean (SD)	0 (0)		0 (0.4)		0 (0.7)		1* (1.2)			
% of control ^b	n/a		n/a		n/a		n/a			
Female (n = 10)										
Mean (SD)	0 (0)		0 (0.4)		0 (0.7)		2* (1.7)			
% of control ^b	n/a		n/a		n/a		n/a			
Doses (mg/kg-d)										

Reference and study design	Results								
	0	0.3	1	3	10	30	100	200	
van der Ven et al. (2006) Rats, Wistar Gavage 28-d exposure starting on PNW 11 Data Quality: ^c High (1.3)	ALT (U/L)								
	Male (n = 3–5)								
	Mean	44.5	40.9	44.3	38.2	45.0	42.7	40.6	39.2
	(SD)	(5.9)	(4.1)	(10.3)	(3.6)	(14.3)	(11.0)	(8.1)	(10.9)
	% of control ^b	–	–8%	0%	–14%	1%	–4%	–9%	–12%
	Female (n = 3–5)								
	Mean	43.4	44.7	39.8	40.5	34.6	38.2	36.0	42.5
	(SD)	(4.6)	(6.5)	(4.5)	(6.7)	(6.6)	(5.0)	(5.2)	(7.5)
	% of control ^b	–	3%	–8%	–7%	–20%	–12%	–17%	–2%
	ALP (U/L)								
Male (n = 3–5)									
Mean	7.34	5.30	3.68	7.43	4.88	5.10	2.74	3.48	
(SD)	(5.59)	(3.66)	(1.82)	(7.43)	(5.75)	(2.54)	(1.61)	(1.95)	
% of control ^b	–	–28%	–50%	1%	–34%	–31%	–63%	–53%	
Female (n = 3–5)**									
Mean	4.66	3.10	4.74	3.72	2.30	2.36	2.73	2.42	
(SD)	(2.91)	(2.76)	(2.50)	(2.14)	(1.21)	(0.33)	(1.55)	(2.71)	
% of control ^b	–	–33%	2%	–20%	–51%	–49%	–41%	–48%	
WIL Research (1997) Rats, Sprague-Dawley Gavage 28-d exposure starting on ~PNW 6 followed by a 14-d recovery period Recovery data not shown	Doses (mg/kg-d)								
		0		125		350		1,000	
	ALT (U/L)								
	Male (n = 6)								
	Mean (SD)	31 (4.9)		23* (5.4)		21* (2.3)		23* (3.5)	
	% of control ^b	–		–26%		–32%		–26%	
	Female (n = 6)								
	Mean (SD)	26 (2.1)		24 (3.7)		27 (3.5)		26 (7.9)	
	% of control ^b	–		–8%		4%		0%	
	ALP (U/L)								
	Male (n = 6)								
	Mean (SD)	199 (40.9)		149 (24.7)		165 (34.6)		154 (37.1)	
	% of control ^b	–		–25%		–17%		–23%	
Female (n = 6)									
Mean (SD)	100 (29.7)		87 (11.8)		85 (20.4)		74 (9.7)		
% of control ^b	–		–13%		–15%		–26%		
AST (U/L)									
Male (n = 6)									
Mean (SD)	80 (18.3)		63* (5.9)		65 (5.4)		61* (6.8)		
% of control ^b	–		–21%		–19%		–24%		

Reference and study design	Results				
Data Quality: ^c High (1.3)	Female (n = 6)				
	Mean (SD)	75 (13.0)	63 (11.5)	61 (9.6)	62 (9.9)
	% of control ^b	–	–16%	–19%	–17%
	GGT (U/L)				
	Male (n = 6)				
	Mean (SD)	1 (0.4)	1 (0.5)	1 (0.5)	1 (0.4)
% of control ^b	–	0%	0%	0%	
Female (n = 6)					
Mean (SD)	1 (0.8)	1 (0.8)	1 (0.9)	1 (0.4)	
% of control ^b	–	0%	0%	0%	
Yanagisawa et al. (2014) Mice, C57BL/6 Males only Gavage Animals dosed once weekly 15-week exposure starting on PNW 6 Dose groups split between standard and high-fat diets Data Quality: ^c Unacceptable (4)*	Doses (µg/kg BW)				
		0	1.75	35	700
	ALT (IU/L), standard diet				
	Male (n = 5–6)				
	Mean (SE)	13.6 (1.04)	15.0 (1.18)	14.2 (1.59)	10.5 (0.22)
	% of control ^b	–	10%	4%	–23%
	ALT (IU/L), high-fat diet				
	Male (n = 5–6)				
	Mean (SE)	34.5 (8.43)	43.0 (15.0)	60.0 (12.2)	61.5 (10.2)
	% of control ^b	–	25%	74%	78%
	AST (IU/L), standard diet				
	Male (n = 5–6)				
Mean (SE)	73.0 (8.86)	74.2 (7.59)	66.6 (6.57)	46.0* (7.96)	
% of control ^b	–	2%	–9%	–37%	
AST (IU/L), high-fat diet					
Male (n = 5–6)					
Mean (SE)	79.7 (7.44)	78.7 (8.58)	101 (8.39)	85.2 (7.50)	
% of control ^b	–	–1%	27%	7%	

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.

**Significant dose response trend as reported by study authors.

^aF1 and F2 offspring presented as mean maternal gestational and lactational F0 and F1 doses, respectively.

^bPercent change compared to control calculated as: $(\text{treated value} - \text{control value}) / \text{control value} \times 100$.

^cTWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PNDs 1–9, and PNDs 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: $100 \text{ ppm} = (8.1 \text{ mg/kg-day} \times 11 \text{ days}) + (14.3 \text{ mg/kg-day} \times 10 \text{ days}) + (21.3 \text{ mg/kg-day} \times 12 \text{ days}) / 33 \text{ days} = 14.8 \text{ mg/kg-day}$.

^dBased on OPPT data evaluation criteria. *[Yanagisawa et al. \(2014\)](#) was scored unacceptable, so it is assigned a score of 4. It's calculated score would have been 1.5

^eBased on OPPT data evaluation criteria

SE = standard error

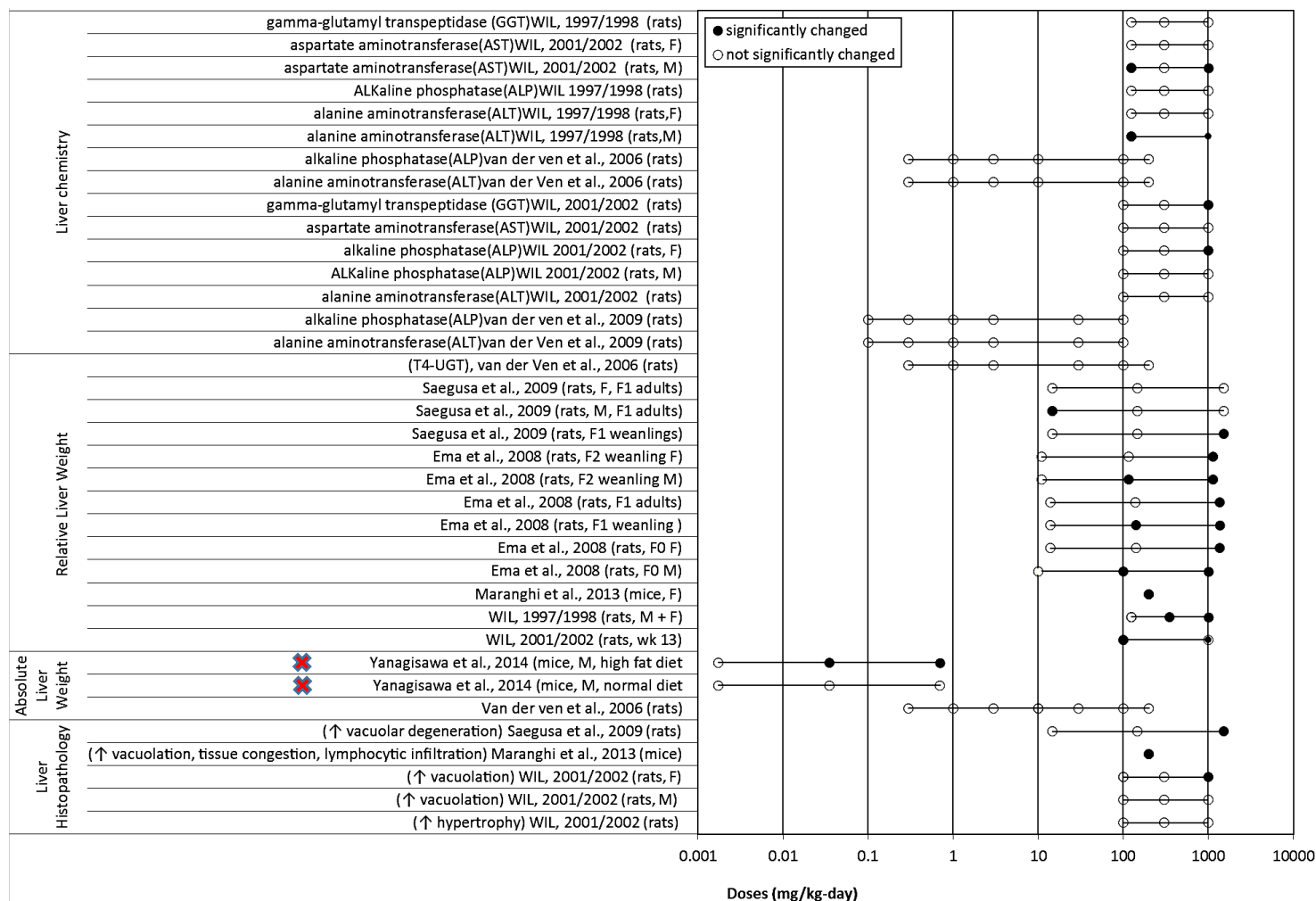


Figure 1-3. Exposure response array of liver effects following oral exposure. All studies scored a High in data quality evaluation except for [Yanagisawa et al. \(2014\)](#), which scored Unacceptable. The study is included only for reference (indicated in the chart by X).

1.2.3 Mechanistic Evidence

Studies have reported a generally consistent pattern of increased liver weight related to HBCD exposure. Increased liver weight is often correlated with induction of hepatic microsomal enzymes, although the level of induction does not necessarily reflect the magnitude of weight change, nor it is a requirement for liver weight increases ([Amacher et al., 1998](#)). HBCD has been shown to induce the expression of several hepatic microsomal enzymes ([Crump et al., 2010](#); [Crump et al., 2008](#); [Germer et al., 2006](#)). Specifically, dose-related increases in liver CYP3A1 and CYP2B1 protein levels were observed in rats exposed to HBCD via diet ([Germer et al., 2006](#)). In addition, dose-related increases in CYP2H1 and CYP3A37 mRNA levels were observed in chicken hepatocytes following in ovo ([Crump et al., 2010](#)) and in vitro exposure ([Crump et al., 2008](#)). Furthermore, some data suggest that induction of hepatic microsomal enzymes responsible for conjugation and elimination of thyroid hormones may contribute to

HBCD-mediated effects related to thyroid perturbation (Section 1.2.1, Mechanistic Evidence). Liver weight changes are also associated with increased hepatocellular hypertrophy and hyperplasia. Hypertrophy was reported in high-dose animals in two studies ([Yanagisawa et al., 2014](#); [WIL Research, 2001](#)); however, hyperplasia was not noted.

HBCD may also impair lipid homeostasis. Several studies observed increased vacuolation in hepatocytes ([Maranghi et al., 2013](#); [Saegusa et al., 2009](#); [WIL Research, 2001, 1997](#)). The only study to evaluate vacuole contents indicated that they predominantly consisted of lipid ([WIL Research, 2001](#)). Chemically-induced impairment of fatty acid metabolism in cells with high energy demands, such as hepatocytes, has been shown to promote accumulation of triglycerides, which form nonmembrane bound vacuoles in cells (i.e., fatty change) ([Wheater and Burkitt, 1996](#)). Various gene expression studies lend supportive evidence for HBCD-mediated disruption of genes involved in lipid metabolism and transport. A 28-day study in rats reported inhibition of peroxisome proliferator-activated receptor (PPAR)-mediated genes involved in lipid metabolism, particularly in females ([Cantón et al., 2008](#)). Statistically significant increases in liver triglyceride levels as well as PPAR-mediated genes involved in lipid metabolism (PPAR γ) and transport (FSp27) were also observed in mice exposed to 0.7 mg/kg-week HBCD while being fed a high-fat diet ([Yanagisawa et al., 2014](#)).

HBCD-mediated alterations in the regulation of lipid metabolism have also been observed in avian species and in vitro. HBCD decreased the mRNA expression of liver fatty acid binding protein in chicken hepatocytes in vitro and following in ovo exposure ([Crump et al., 2010](#); [Crump et al., 2008](#)). The observed effects on lipid homeostasis may be a direct effect or secondary to perturbation of thyroid function. In humans and animal models, hypothyroidism is thought to be associated with altered liver metabolism and increased triglycerides and cholesterol, as well as non-alcoholic fatty liver disease ([Eshraghian and Jahromi, 2014](#); [Pucci et al., 2000](#)). HBCD studies that evaluated serum lipid profiles did not report any significant changes in serum cholesterol or triglyceride levels in exposed rats ([van der Ven et al., 2006](#); [WIL Research, 2001](#)) or mice ([Yanagisawa et al., 2014](#)) fed a standard diet; however, statistically significant increases in levels of liver triglycerides were reported in mice exposed concurrently to HBCD and a high-fat diet ([Yanagisawa et al., 2014](#)).

The lack of increased incidence of necrosis or apoptosis and/or serum enzymatic markers of hepatocellular damage suggests that HBCD is not highly cytotoxic. However, there is evidence to suggest the exposure to HBCD can increase the production of reactive oxygen species (ROS). Dose-related increases in ROS were observed in human hepatocyte and carcinoma cell lines following in vitro exposures ([An et al., 2013](#); [Hu et al., 2009b](#)).

1.3 Reproductive Effects

1.3.1 Female Reproductive Effects

1.3.1.1 Human Evidence

The potential for HBCD to affect the female reproductive system has not been investigated in humans.

1.3.1.2 Animal Evidence

Evidence to inform the potential for HBCD to induce female reproductive effects comes from five studies in rats ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#); [WIL Research, 2001, 1997](#)) and one study in mice ([Maranghi et al., 2013](#)) with exposure durations ranging from 28 days to two generations. Endpoints evaluated in these studies include fertility and pregnancy outcomes, hormone levels, markers of reproductive differentiation and development, and reproductive organ weights. Evidence pertaining to female reproductive effects in experimental animals following oral exposure to HBCD is summarized in Table 1-4 and Figure 1-4. Effect categories with stronger evidence are presented first, with individual studies ordered by study duration and then species. If not otherwise indicated, endpoint measurements were made in adults.

Fertility and pregnancy outcomes were evaluated in three rat studies ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#)). Dose-related decreases in pregnancy incidence in the F0 and F1 dams was reported in the two-generation reproductive toxicity study using doses up to approximately 1,300 mg/kg-day HBCD ([Ema et al., 2008](#)). In the F1 females, a 36–37% decrease in the number of primordial follicles was reported at approximately 140 mg/kg-day HBCD or greater received throughout gestation, lactation, and adulthood ($p < 0.05$) ([Ema et al., 2008](#)). This endpoint was only evaluated in the F1 females. The one-generation reproductive toxicity study, using doses up to 100 mg/kg-day HBCD, reported no significant trend in successful matings, defined as the rate of matings resulting in offspring ([van der Ven et al., 2009](#)). The results from [van der Ven et al. \(2009\)](#) are not directly comparable to the findings of [Ema et al. \(2008\)](#) due to the low doses used by investigators (i.e., a dose range lower than doses associated with effects in [Ema et al. \(2008\)](#)). Incidence of pregnancy was not measured in the developmental study using doses up to approximately 1,500 mg/kg-day HBCD because the study began with previously impregnated females ([Saegusa et al., 2009](#)). Other measures of fertility and pregnancy outcomes (e.g., gestational duration, number of implantation sites, litter size) reported in these three studies showed no effect with HBCD exposure studies ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#)).

HBCD-induced changes in reproductive hormone concentrations were examined in both rats ([Ema et al., 2008](#)) and mice ([Maranghi et al., 2013](#)). [Ema et al. \(2008\)](#) observed elevated follicle-stimulating hormone (FSH) concentrations (41%) only in F0 rats exposed to approximately 1,300 mg/kg-day; serum levels of estradiol, testosterone, progesterone, and luteinizing hormone (LH) were not affected. Statistically significant increases in serum testosterone levels (57%) were reported in female mice exposed to 199 mg/kg-day for 28 days ([Maranghi et al., 2013](#)), resulting in a 56% elevation in the testosterone/17 β -estradiol ratio.

Effects on reproductive differentiation and development were evaluated in three studies in rats ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#)). Although [van der Ven et al. \(2009\)](#) reported a dose-related delay in vaginal opening, a measurement of puberty onset, at concentrations up to 100 mg/kg-day, no treatment-related effects were observed in the other two studies that used concentrations up to 1,505 mg/kg-day ([Saegusa et al., 2009](#); [Ema et al., 2008](#)). There were no HBCD-mediated effects on anogenital distance (AGD) ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#)).

Treatment-related effects on female reproductive organ weights were evaluated in six studies using both rats ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#); [WIL Research, 2001, 1997](#)) and mice ([Maranghi et al., 2013](#)). Absolute uterine weights were decreased by 17–23% in a 90-day oral study in rats ([WIL Research, 2001](#)), but the decreases were not dose-related and returned to control levels after a 4-week recovery period. Absolute, but not relative, uterine weight showed a statistically significant decrease (22%) in F2 rats (PND 26) in the high-dose group (approximately 1,300 mg/kg-day) ([Ema et al., 2008](#)); no exposure-related effects on uterine weight were observed in F1 animals. No other clear treatment-related effects were observed on absolute or relative uterine ([Maranghi et al., 2013](#); [Saegusa et al., 2009](#); [van der Ven et al., 2009](#)) or ovary weights ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#); [WIL Research, 2001, 1997](#)).

Table 1-4. Evidence pertaining to female reproductive effects in animals following exposure to HBCD

Reference and study design	Results									
<i>Fertility and pregnancy outcomes</i>										
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Data Quality: ^d High (1.0)	Doses (mg/kg-d)									
	Female, F0	0	14	141	1,363					
	Female, F1	0	14	138	1,363					
	Incidence of pregnant females									
	Female, F0 (n = 23–24)									
	Incidence	24/24	22/24	20/24	19/23					
	Female, F1 (n = 21–24)									
	Incidence	23/24	23/24	21/24	21/24					
	Primordial follicles (count)									
	Female, F1 (n = 10)									
Mean (SD)	316.3 (119.5)	294.2 (66.3)	197.9* (76.9)	203.4* (79.5)						
% of control ^a	–	–7%	–37%	–36%						
Other pregnancy outcomes										
No dose-related changes in other outcomes (e.g., number of implantation sites, gestation duration, litter size) reported in either generation										
van der Ven et al. (2009) Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation;	Doses (mg/kg-d)									
		0	0.1	0.3	1	3	10	30	100	
	Successful matings									
	Female, F0 (n = 8–10)									
	Incidence	8/10	8/10	4/10	7/10	8/10	6/8	6/10	6/10	
Other pregnancy outcomes										

Reference and study design	Results																																																																																
dietary exposure post weaning through PNW 11 Data Quality: ^d High (1.0)	No significant dose-response trend in other outcomes (e.g., number of implantation sites, gestation duration, litter size)																																																																																
Saegusa et al. (2009) Crj:CD(SD)IGS, rat Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11 Data Quality: ^d High (1.2)	<table border="1"> <tr> <td colspan="6" data-bbox="477 464 1421 506">Doses (mg/kg-d)^c</td> </tr> <tr> <td></td> <td data-bbox="760 506 781 537" style="text-align: center;">0</td> <td data-bbox="938 506 959 537" style="text-align: center;">15</td> <td data-bbox="1117 506 1138 537" style="text-align: center;">146</td> <td data-bbox="1295 506 1317 537" style="text-align: center;">1,505</td> <td colspan="1"></td> </tr> <tr> <td colspan="6" data-bbox="477 548 1421 590">Pregnancy outcomes</td> </tr> <tr> <td colspan="6" data-bbox="477 590 1421 842"> No dose-related effect on pregnancy outcomes (e.g., number of implantation sites, gestation duration, litter size) </td> </tr> </table>					Doses (mg/kg-d)^c							0	15	146	1,505		Pregnancy outcomes						No dose-related effect on pregnancy outcomes (e.g., number of implantation sites, gestation duration, litter size)																																																									
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<i>Hormonal measures</i>																																																																																	
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Data Quality: ^d High (1.0)	<table border="1"> <tr> <td colspan="6" data-bbox="477 884 1421 926">Doses (mg/kg-d)</td> </tr> <tr> <td></td> <td data-bbox="732 926 753 957" style="text-align: center;">0</td> <td data-bbox="911 926 932 957" style="text-align: center;">14</td> <td data-bbox="1089 926 1110 957" style="text-align: center;">141</td> <td data-bbox="1268 926 1289 957" style="text-align: center;">1,363</td> <td colspan="1"></td> </tr> <tr> <td></td> <td data-bbox="732 957 753 989" style="text-align: center;">0</td> <td data-bbox="911 957 932 989" style="text-align: center;">14</td> <td data-bbox="1089 957 1110 989" style="text-align: center;">138</td> <td data-bbox="1268 957 1289 989" style="text-align: center;">1,363</td> <td colspan="1"></td> </tr> <tr> <td colspan="6" data-bbox="477 999 1421 1041">FSH (ng/mL)</td> </tr> <tr> <td colspan="6" data-bbox="477 1041 1421 1083">Female, F0 (n = 8)</td> </tr> <tr> <td></td> <td data-bbox="505 1083 553 1115">Mean (SD)</td> <td data-bbox="683 1083 797 1115">4.17 (0.51)</td> <td data-bbox="862 1083 976 1115">4.84 (0.63)</td> <td data-bbox="1040 1083 1154 1115">4.88 (1.05)</td> <td data-bbox="1219 1083 1333 1115">5.86* (1.11)</td> </tr> <tr> <td></td> <td data-bbox="505 1115 634 1146">% of control^a</td> <td data-bbox="732 1115 753 1146" style="text-align: center;">–</td> <td data-bbox="894 1115 943 1146" style="text-align: center;">16%</td> <td data-bbox="1057 1115 1105 1146" style="text-align: center;">17%</td> <td data-bbox="1219 1115 1268 1146" style="text-align: center;">41%</td> </tr> <tr> <td colspan="6" data-bbox="477 1146 1421 1188">Female, F1 (n = 8)</td> </tr> <tr> <td></td> <td data-bbox="505 1188 553 1220">Mean (SD)</td> <td data-bbox="683 1188 797 1220">5.89 (1.60)</td> <td data-bbox="862 1188 976 1220">6.07 (0.60)</td> <td data-bbox="1040 1188 1154 1220">6.33 (0.82)</td> <td data-bbox="1219 1188 1333 1220">6.52 (0.95)</td> </tr> <tr> <td></td> <td data-bbox="505 1220 634 1251">% of control^a</td> <td data-bbox="732 1220 753 1251" style="text-align: center;">–</td> <td data-bbox="894 1220 932 1251" style="text-align: center;">3%</td> <td data-bbox="1057 1220 1094 1251" style="text-align: center;">7%</td> <td data-bbox="1219 1220 1256 1251" style="text-align: center;">11%</td> </tr> <tr> <td colspan="6" data-bbox="477 1262 1421 1304">Other hormone measurements</td> </tr> <tr> <td colspan="6" data-bbox="477 1304 1421 1419"> Exposure-related changes were not found for progesterone, LH, or estradiol in the F0 and F1 females. </td> </tr> </table>					Doses (mg/kg-d)							0	14	141	1,363			0	14	138	1,363		FSH (ng/mL)						Female, F0 (n = 8)							Mean (SD)	4.17 (0.51)	4.84 (0.63)	4.88 (1.05)	5.86* (1.11)		% of control ^a	–	16%	17%	41%	Female, F1 (n = 8)							Mean (SD)	5.89 (1.60)	6.07 (0.60)	6.33 (0.82)	6.52 (0.95)		% of control ^a	–	3%	7%	11%	Other hormone measurements						Exposure-related changes were not found for progesterone, LH, or estradiol in the F0 and F1 females.									
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Maranghi et al. (2013) Mice, BALB/c Females only Diet 28-d exposure starting on PND 26 Data Quality: ^d High (1.3)	<table border="1"> <tr> <td colspan="6" data-bbox="477 1419 1421 1461">Doses (mg/kg-d)</td> </tr> <tr> <td></td> <td data-bbox="824 1461 846 1493" style="text-align: center;">0</td> <td colspan="3"></td> <td data-bbox="1198 1461 1219 1493" style="text-align: center;">199</td> </tr> <tr> <td colspan="6" data-bbox="477 1493 1421 1535">Testosterone (ng/mL)</td> </tr> <tr> <td colspan="6" data-bbox="477 1535 1421 1577">Female (n = 10)</td> </tr> <tr> <td></td> <td data-bbox="505 1577 553 1608">Mean (SD)</td> <td data-bbox="764 1577 878 1608" style="text-align: center;">0.07 (0.02)</td> <td colspan="3"></td> <td data-bbox="1154 1577 1268 1608" style="text-align: center;">0.11* (0.07)</td> </tr> <tr> <td></td> <td data-bbox="505 1608 634 1640">% of control^a</td> <td data-bbox="732 1608 753 1640" style="text-align: center;">–</td> <td colspan="3"></td> <td data-bbox="1187 1608 1235 1640" style="text-align: center;">57%</td> </tr> <tr> <td colspan="6" data-bbox="477 1640 1421 1682">Testosterone/estradiol</td> </tr> <tr> <td colspan="6" data-bbox="477 1682 1421 1724">Female (n = 10)</td> </tr> <tr> <td></td> <td data-bbox="505 1724 553 1755">Mean (SD)</td> <td data-bbox="781 1724 878 1755" style="text-align: center;">8.5 (2.1)</td> <td colspan="3"></td> <td data-bbox="1154 1724 1268 1755" style="text-align: center;">13.3* (6.7)</td> </tr> <tr> <td></td> <td data-bbox="505 1755 634 1787">% of control^a</td> <td data-bbox="732 1755 753 1787" style="text-align: center;">–</td> <td colspan="3"></td> <td data-bbox="1187 1755 1235 1787" style="text-align: center;">56%</td> </tr> <tr> <td colspan="6" data-bbox="477 1787 1421 1829">Other hormone measurements</td> </tr> <tr> <td colspan="6" data-bbox="477 1829 1421 1877"> Exposure-related changes were not found for estradiol. </td> </tr> </table>					Doses (mg/kg-d)							0				199	Testosterone (ng/mL)						Female (n = 10)							Mean (SD)	0.07 (0.02)				0.11* (0.07)		% of control ^a	–				57%	Testosterone/estradiol						Female (n = 10)							Mean (SD)	8.5 (2.1)				13.3* (6.7)		% of control ^a	–				56%	Other hormone measurements						Exposure-related changes were not found for estradiol.					
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	% of control ^a	–				56%																																																																											
Other hormone measurements																																																																																	
Exposure-related changes were not found for estradiol.																																																																																	

Reference and study design	Results								
<i>Reproductive differentiation and development</i>									
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Data Quality: ^d High (1.0)	Doses (mg/kg-d)								
	F1 offspring^d	0	17	168	1,570				
	F2 offspring^d	0	15	139	1,360				
	Time to vaginal opening (d)								
	Female F1 (n = 24)								
	Mean (SD)	30.9 (2.0)	30.3 (2.6)	30.1 (1.8)	30.8 (2.2)				
% of control ^a	-	-2%	-3%	0%					
AGD (mm)									
No dose-related changes in the F1 or F2 female pups									
van der Ven et al. (2009) Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Data Quality: ^d High (1.2)	Doses (mg/kg-d)								
		0	0.1	0.3	1	3	10	30	100
	Time to vaginal opening (days)								
	Female, F1 (n = 4–5)^{b **}								
	Mean (SD)	35.4 (2.3)	35.3 (2.2)	36.2 (2.4)	36.8 (4.1)	36.8 (3.3)	35.4 (2.7)	34.8 (1.6)	39.9 (2.6)
	% of control ^a	-	0%	2%	4%	4%	0%	-2%	13%
AGD (mm)									
No significant dose-response trend									
Saegusa et al. (2009) Crj:CD(SD)IGS, rat Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11 Data Quality: ^d High (1.3)	Doses (mg/kg-d)^c								
		0	15	146	1,505				
	Time to vaginal opening (d)								
	Female F1 (n = 12–14)								
	Mean (SD)	35.4 (1.9)	35.6 (1.8)	34.9 (1.7)	34.4 (2.1)				
	% of control ^a	-	1%	-1%	-3%				
AGD (mm)									
No dose-related change									
<i>Reproductive organ weights</i>									

Reference and study design	Results				
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Data Quality: ^d High (1.0)	Doses (mg/kg-d)				
	F1 offspring^d	0	17	168	1,570
	Female F1 adult	0	14	138	1,363
	F2 offspring^d	0	15	139	1,360
	Absolute ovary weight (mg)				
	Female, F1, PND 26 (n = 14–23)				
	Mean (SD)	20.8 (3.7)	22.8 (3.6)	21.0 (4.0)	20.9 (3.4)
	% of control ^a	–	10%	1%	0%
	Female, F1, adult (n = 13–22)				
	Mean (SD)	102.4 (12.9)	106.4 (13.2)	108.6 (18.0)	104.9 (16.9)
	% of control ^a	–	4%	6%	2%
	Female, F2, PND 26 (n = 13–21)				
	Mean (SD)	20.0 (3.9)	22.9* (2.6)	20.9 (3.9)	18.2 (4.0)
	% of control ^a	–	14%	4%	–9%
	Relative ovary weight (mg/100 g BW)				
	Female, F1, PND 26 (n = 14–23)				
	Mean (SD)	26.5 (4.5)	27.5 (4.1)	25.0 (3.8)	28.9 (3.7)
	% of control ^a	–	4%	–6%	9%
	Female, F1, adult (n = 13–22)				
	Mean (SD)	31.8 (4.2)	32.6 (3.9)	33.1 (5.3)	34.1 (4.2)
	% of control ^a	–	3%	4%	7%
	Female, F2, PND 26 (n = 13–21)				
	Mean (SD)	26.9 (5.1)	30.5* (3.9)	28.8 (4.2)	32.1* (7.5)
% of control ^a	–	13%	7%	19%	
Absolute uterus weight (mg)					
Female, F1, PND 26 (n = 14–23)					
Mean (SD)	57.0 (10.9)	62.0 (14.1)	64.1 (18.6)	51.9 (12.4)	
% of control ^a	–	9%	12%	–9%	
Female, F1, adult (n = 13–22)					
Mean (SD)	966 (216)	913 (188)	955 (204)	949 (156)	
% of control ^a	–	–5%	–1%	–2%	
Female, F2, PND 26 (n = 13–21)					
Mean (SD)	60.8 (16.1)	63.6 (15.1)	57.0 (15.7)	47.6* (11.4)	
% of control ^a	–	5%	–6%	–22%	
Relative uterus weight (mg/100 g BW)					
Female, F1, PND 26 (n = 14–23)					
Mean (SD)	73.6 (17.5)	74.9 (17.7)	76.0 (18.4)	71.9 (16.2)	
% of control ^a	–	2%	3%	–2%	
Female, F1, adult (n = 13–22)					
Mean (SD)	299 (64)	282 (65)	291 (64)	313 (69)	
% of control ^a	–	–6%	–3%	5%	
Female, F2, PND 26 (n = 13–21)					

Reference and study design	Results								
	Mean (SD)	80.9 (16.3)	84.4 (21.0)	78.7 (21.7)	83.7 (20.3)				
	% of control ^a	–	4%	–3%	3%				
van der Ven et al. (2009)	Doses (mg/kg-d)								
		0	0.1	0.3	1	3	10	30	100
Rats, Wistar Diet One generation	Absolute ovary weight (left and right) (g)								
	Female, F1, PNW 11 (n = 4–5)								
	Mean (SD)	0.10 (0.01)	0.13 (0.02)	0.11 (0.02)	0.11 (0.003)	0.13 (0.02)	0.11 (0.02)	0.12 (0.02)	0.11 (0.02)
F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating	% of control ^a	–	21%	11%	9%	24%	8%	17%	7%
F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	Absolute uterus weight (g)								
	Female, F1, PNW 11 (n = 4–5)								
	Mean (SD)	0.53 (0.11)	0.60 (0.20)	0.50 (0.11)	0.75 (0.38)	0.71 (0.39)	0.94 (0.28)	0.48 (0.10)	0.49 (0.22)
	% of control ^a	–	13%	–6%	42%	34%	77%	–9%	–8%
Data Quality: ^d High (1.2)									
WIL Research (2001)	Doses (mg/kg-d)								
		0	100	300	1,000				
Rats, Crl:CD(SD)IGS BR Gavage 90 d exposure starting on ~PNW 7 followed by a 28-d recovery period	Absolute ovary with oviduct weight (g)								
	Female (n = 10)								
	Mean (SD)	0.14 (0.03)	0.13 (0.03)	0.13 (0.03)	0.13 (0.03)	0.13 (0.03)	0.15 (0.02)		
	% of control ^a	–	–10%	–9%	–9%	–9%	3%		
Recovery data not shown	Relative ovary with oviduct weight (g/100 g BW)								
	Female (n = 10)								
	Mean (SD)	0.05 (0.01)	0.05 (0.01)	0.05 (0.01)	0.05 (0.01)	0.05 (0.01)	0.05 (0.01)		
	% of control ^a	–	–8%	–8%	–12%	–12%	2%		
Data Quality: ^d High (1.0)	Absolute uterus with cervix weight (g)								
	Female (n = 10)								
	Mean (SD)	0.81 (0.25)	0.64 (0.16)	0.67 (0.14)	0.67 (0.14)	0.67 (0.14)	0.62 (0.17)		
	% of control ^a	–	–21%	–17%	–17%	–17%	–23%		
	Relative uterus with cervix weight (g/100 g BW)								
	Female (n = 10)								
	Mean (SD)	0.29 (0.07)	0.23 (0.05)	0.23 (0.05)	0.22 (0.04)	0.22 (0.04)	0.22 (0.07)		
	% of control ^a	–	–20%	–20%	–21%	–21%	–23%		
WIL Research (1997)	Doses (mg/kg-d)								
		0	125	350	1,000				
Rats, Sprague-Dawley Gavage	Relative ovary with oviduct weight (g/100 g BW)								
	Female (n = 6)								

Reference and study design	Results				
28-d exposure starting on ~PNW 6 followed by a 14-d recovery period Recovery data not shown Data Quality: ^d High (1.3)	Mean (SD)	0.06 (0.0003)	0.06 (0.01)	0.06 (0.01)	0.06 (0.01)
	% of control ^a	-	0%	0%	0%
Saegusa et al. (2009) Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11 Data Quality: ^d High (1.2)	Doses (mg/kg-d) ^d	0	15	146	1,505
	Relative ovary weight (mg/100 g BW)				
	Female, F1, PND 20 (n = 10)				
	Mean (SD)	32.3 (3.9)	30.9 (4.9)	28.1 (6.3)	28.7 (3.4)
	% of control ^a	-	-4%	-13%	-11%
	Female, F1, PNW 11 (n = 10)				
	Mean (SD)	31.8 (6.1)	32.8 (2.6)	32.2 (5.7)	34.0 (4.8)
	% of control ^a	-	3%	1%	7%
	Relative uterus weight (g/100 g BW)				
	Female, F1, PND 20 (n = 10)				
	Mean (SD)	0.08 (0.01)	0.08 (0.01)	0.08 (0.01)	0.07 (0.01)
	% of control ^a	-	0%	-4%	-9%
	Female, F1, PNW 11 (n = 10)				
	Mean (SD)	0.16 (0.04)	0.15 (0.02)	0.16 (0.02)	0.17 (0.03)
	% of control ^a	-	-6%	0%	6%
Maranghi et al. (2013) Mice, BALB/c Females only Diet 28-d exposure starting on PND 26 Data Quality: ^d High (1.3)	Doses (mg/kg-d)	0		199	
	Absolute uterus weight (g)				
	Female (n = 10-15)				
	Mean (SD)	0.140 (0.051)		0.141 (0.041)	
	% of control ^a	-		1%	
	Relative uterus weight (%)				
	Female (n = 10-15)				
	Mean (SD)	0.66 (0.24)		0.71 (0.21)	
	% of control ^a	-		8%	

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.

**Significant dose response trend as reported by study authors.

^aPercent change compared to control calculated as: (treated value - control value)/control value × 100.

^bExact number of animals examined per dose group was unclear in the published paper.

^cTWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10-20, PNDs 1-9, and PNDs 9-20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = (8.1 mg/kg-day × 11 days) + (14.3 mg/kg-day × 10 days) + (21.3 mg/kg-day × 12 days)/33 days = 14.8 mg/kg-day.

^dF1 and F2 offspring doses presented as maternal F0 and F1 mean gestational and lactational doses, respectively.

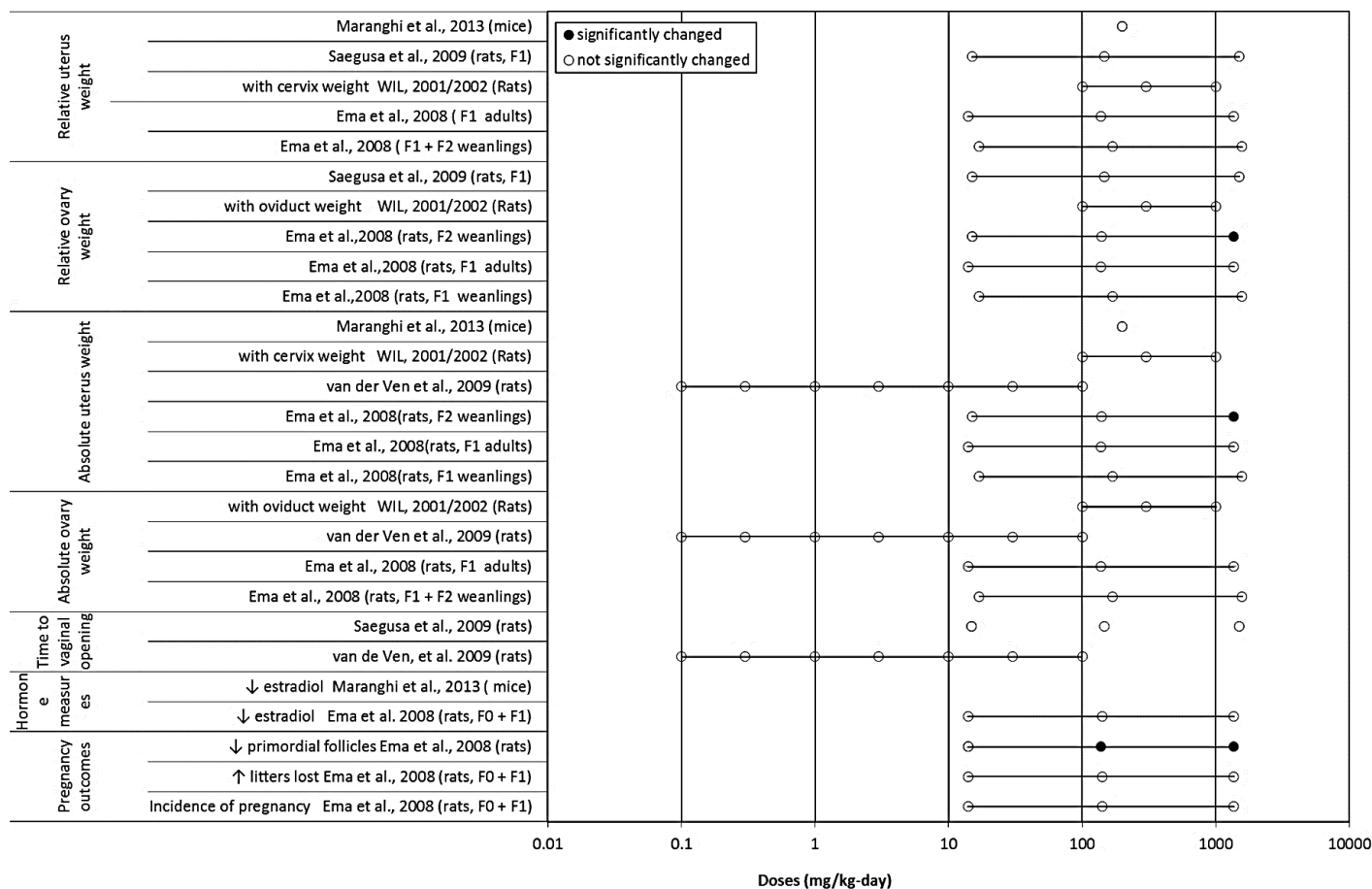


Figure 1-4. Exposure response array of female reproductive system effects following oral exposure. All studies scored a High in data quality evaluation.

1.3.1.3 Mechanistic Evidence

The available mechanistic evidence related to HBCD-mediated effects on the reproductive system is focused on dysregulation of reproductive hormone homeostasis.

Human and rodent cell culture models provide some evidence to support the potential for HBCD to alter the function of several reproductive hormones. Human breast cancer cells (MDA-kb2) co-exposed with dihydroxytestosterone, HBCD potentiated expression of androgen-receptor mediated genes, but did not act as a direct AR agonist (Christen et al., 2010). In human prostate cancer cells (LNCaP), however, HBCD treatment elicited a pattern of responses that is characteristic of AR activation (e.g., increased cell migration and viability, and reduction of apoptotic markers), but at a lower potency than the endogenous ligand (Kim et al., 2016). FSH was also affected in rat granulosa and leydig cells; HBCD altered FSH- and LH-mediated signaling pathways (Fa et al., 2015; Fa et al., 2014). Effects on the estrogen receptor are less consistent. Assay findings using human breast cancer cells (T47D and MCF-7) indicated that HBCD may act as an estrogen antagonist (Krivoshiev et al., 2016; Hamers et al., 2006); however, these findings were not consistent with other studies that used one of the same breast

cancer cell lines (MCF-7) or ovarian cancer cells ([Kang et al., 2012](#); [Park et al., 2012](#); [Dorosh et al., 2011](#); [Yamada-Okabe et al., 2005](#)).

In addition to hormone receptor level effects, several studies indicate that HBCD may also perturb enzymes involved in the synthesis and metabolism of reproductive hormones. In female rats, HBCD exposure increased mRNA and protein levels as well as activity of the CYP3A family of enzymes ([Cantón et al., 2008](#); [Germer et al., 2006](#)), which play an important role in the metabolism and excretion of estrogens ([Kretschmer and Baldwin, 2005](#)). Studies in rat primary Leydig and human adrenocortical carcinoma cell lines indicate that HBCD exposure may interfere with activity and/or cell signaling pathways of several enzymes involved in steroid synthesis ([Scott et al., 2009](#); [Cantón et al., 2006](#)), including CYP17 ([Fa et al., 2013](#); [Fernandez Canton et al., 2005](#)) and CYP19A1 ([van den Dungen et al., 2015](#)), CYP11A1, and HSD17 β ([Fa et al., 2015](#)).

1.3.2 Male Reproductive Effects

1.3.2.1 Human Evidence

Epidemiological studies evaluating HBCD exposure and reproductive endpoints include a birth cohort ([Meijer et al., 2012](#)) and a cross-sectional study of male infertility patients ([Johnson et al., 2013](#)) (Table 1-5). The birth cohort study in the Netherlands examined maternal serum HBCD levels in relation to male infants' testes volume and penile length at 3 and 18 months (n = 44) as well as steroidal and gonadotropin hormone levels at 3 months (n = 34) ([Meijer et al., 2012](#)). Effect estimates for the association with testes volume or penile length were not provided but were not reported to be statistically significant. A weak to moderate correlation coefficient ($r = -0.31$; $0.05 < p < 0.10$) was observed between maternal serum HBCD and free testosterone. No other effects on steroidal or gonadotropin hormones were associated with serum HBCD levels (effect estimates not provided). A study examining the relationship between HBCD concentrations in household dust and reproductive hormones in 38 adult men from the United States attending an infertility clinic ([Johnson et al., 2013](#)) reported statistically significant correlations for decreased sex hormone binding globulin (SHBG) ($r = -0.35$; $p = 0.03$) and increased free androgen index (testosterone/SHBG) ($r = 0.46$; $p = 0.004$); the effect on the free androgen index was likely due to decreased SHBG levels, as testosterone concentrations did not appear to be related to HBCD exposure. Correlation coefficients for other hormones were not reported, but were described as not statistically significant ([Johnson et al., 2013](#)).

The available evidence for an association between HBCD exposure and male reproductive effects in humans is insufficient. Two epidemiological studies that evaluated male reproductive outcomes (see Table 1-5) provided limited evidence of male reproductive effects (effects on serum testosterone and SHGC levels) associated with HBCD exposure in humans.

1.3.2.2 Animal Evidence

Evidence to inform the potential for HBCD to induce male reproductive effects, including reproductive differentiation and development, spermatogenic measures, and reproductive organ weights, comes from five studies in rats ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#); [van der Ven et al., 2006](#); [WIL Research, 2001](#)) with exposure durations ranging from 28 days to two generations. Evidence pertaining to male reproductive effects in experimental

animals following oral exposure to HBCD is summarized in Table 1-6 and Figure 1-5. Effect categories with stronger evidence are presented first, with individual studies ordered by study duration and then species. If not otherwise indicated, endpoint measurements were made in adults.

The available evidence for an association between HBCD exposure and male reproductive effects in experimental animals is insufficient for drawing conclusions (Table 1-6). One study found a significant dose-related increase in AGD, a measure of reproductive differentiation and development, only on PND 4 ([van der Ven et al., 2009](#)) and the biological significance of increased AGD is unclear. [van der Ven et al. \(2009\)](#) also reported a significant trend with dose for epididymal sperm with separate heads in rats continuously exposed to HBCD from gestation through PNW 11, but not after a 28-day exposure in adults ([van der Ven et al., 2006](#)). Statistically significant increases (9–12% relative to control) in relative testis weight were reported for PND 26 F1 rats in all three dose groups (approximately 17–1,500 mg/kg-day) in a two-generation reproductive study ([Ema et al., 2008](#)), but not in 15-week F1 males or PND 26 F2 males in the same study. Relative testes weights in HBCD-exposed rats were increased (6–7%) in [WIL Research \(2001\)](#) and decreased (4–7%) in [Saegusa et al. \(2009\)](#); in both studies, changes were not statistically significantly different. Two studies reported statistically significant changes in relative prostate weight in high-dose animals; however, the direction of the effect was not consistent across studies, with [Ema et al. \(2008\)](#) reporting a decrease and [WIL Research \(2001\)](#) reporting an increase. Furthermore, this effect was no longer present following a 4-week recovery period ([WIL Research, 2001](#)). No other dose-related effects were observed for other measures of male reproductive differentiation and development ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#)), spermatogenic measures ([van der Ven et al., 2009](#); [Ema et al., 2008](#); [van der Ven et al., 2006](#); [WIL Research, 2001](#)), or male reproductive organ weights ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#); [WIL Research, 2001](#)).

Table 1-5. Evidence pertaining to male reproductive toxicity of HBCD in humans

Reference and study design	Results
<p>Meijer et al. (2012) (the Netherlands, COMPARE cohort, 2001–2002) Population: Birth cohort, 90 singleton, term births, 55 healthy boys, assessed at 3 mo (n = 55) and 18 mo (n = 52); 44 with HBCD measures, 45 with hormone measures, 34 with both measures Exposure measures: Prenatal exposure, maternal serum at 35th week of pregnancy 1,2,5,6,9,10-HBCD (HBCD) detected in 43 of 44 samples LOD 0.8 pg/g serum; LOQ = 9 pg/g serum Median 0.7 (range: <LOD–7.4) ng/g lipid Effect measures: Reproductive hormones (serum, collected at 3 mo) (immunoassay details in immunoassay details in Laven et al., 2004)</p> <ul style="list-style-type: none"> • testosterone • SHBG • FSH • LH • estradiol 	<p>Spearman correlation between HBCD in maternal serum and free testosterone: $r = -0.31$ ($0.05 < p\text{-value} < 0.10$).</p> <p>Correlations with other hormones noted as not statistically significant, but effect estimates were not reported.</p> <p>No significant correlations between prenatal exposure to HBCD and testes volume or penile length were found (data not shown).</p>

Reference and study design	Results
<ul style="list-style-type: none"> inhibin B <p>Testes volume, measured by ultrasound (ages 3 and 18 mo); penile length (ages 3 and 18 mo) Analysis: Spearman correlation</p> <p>Data quality:^a Medium (1.9)</p>	
<p>Johnson et al. (2013) (USA, 2002–2003) Population: 38 men (18–54 yrs old), from couples seeking infertility treatment; approximately 65% participation into general study; participation rate in the vacuum bag collection phase not reported Exposure measures: HBCD exposure from vacuum bag dust; three main stereoisomers of HBCD presented together; HBCD detected in 97% of samples; LOD not reported; median 246 ng/g dust (90th percentile 1,103 ng/g dust) Effect measures: Non-fasting blood sample (immunoassay details in immunoassay details in Meeker et al., 2008) testosterone Sex hormone binding globulin (SHBG) Follicle stimulating hormone (FSH) Luteinizing hormone (LH) estradiol inhibin B prolactin Analysis: All variables analyzed as continuous variables; Spearman’s correlation between HBCD in house dust and serum hormone levels; multivariable models adjusted for age and BMI, but results for HBCD model results not reported</p> <p>Data quality:^a High (1.6)</p>	<p>Spearman r (<i>p</i>-value)</p> <p>Free androgen index (testosterone/SHBG) 0.46 (<i>p</i> = 0.004)</p> <p>SHBG -0.35^a (<i>p</i> = 0.03)</p> <p>Multivariate models adjusted for age and BMI reportedly produced similar results to the bivariate results (data not reported for HBCD).</p> <p>Results for other hormones not shown.</p> <p>Note that HBCD was not strongly correlated with other flame retardants measured (Spearman correlation coefficients ranging from -0.20 to 0.27, all <i>p</i>-values > 0.10)</p>

^a Based on OPPT data evaluation criteria

Table 1-6. Evidence pertaining to male reproductive effects in animals following exposure to HBCD

Reference and study design	Results									
<i>Reproductive differentiation and development</i>										
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Data quality:^c High (1.0)	Doses (mg/kg-d)									
	F1 offspring^a		0	17	168	1,570				
	F2 offspring^a		0	15	139	1,360				
	AGD (mm)									
	Male, F1, PND 4 (n = 18–24 litters)									
	Mean (SD)	5.37 (0.41)	5.44 (0.36)	5.38 (0.32)	5.20 (0.51)					
% change ^b	–	1%	0%	–3%						
Male, F2, PND 4 (n = 19–22 litters)										
Mean (SD)	5.12 (0.54)	5.12 (0.41)	5.04 (0.42)	4.84 (0.39)						
% change ^b	–	0%	–2%	–5%						
van der Ven et al. (2009) Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Data quality:^c High (1.0)	Doses (mg/kg-d)									
			0	0.1	0.3	1	3	10	30	100
	AGD (mm)									
	Male, F1, PND 4 (n ≥ 14)^{c **}									
	Mean (SD)	4.6 (0.8)	5.1 (1.1)	4.7 (0.8)	4.8 (1.0)	5.0 (0.8)	5.0 (0.9)	4.5 (0.8)	5.4 (1.0)	
	% change ^b	–	11%	2%	4%	9%	9%	–2%	17%	
	Male, F1, PND 7 (n ≥ 14)^c									
	Mean (SD)	6.2 (1.2)	6.7 (1.2)	5.5 (1.1)	6.4 (1.4)	6.1 (1.3)	6.0 (1.3)	6.6 (1.0)	6.3 (1.2)	
	% change ^b	–	8%	–11%	3%	–2%	–3%	6%	2%	
	Male, F1, PND 21 (n ≥ 14)^c									
Mean (SD)	19.0 (6.0)	19.1 (4.1)	14.8 (2.6)		18.7 (2.9)	18.3 (5.5)	18.9 (6.1)	16.0 (2.2)		
% change ^b	–	1%	–22%	n/a	–2%	–4%	–1%	–16%		
Value for male F1 PND 21 rats at 1 mg/kg-d was “n/a” in study report.										
Saegusa et al. (2009) Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11 Data quality:^c	Doses (mg/kg-d)^d									
			0	15	146	1,505				
	AGD (mm)									
	Male, F1, PND 1 (n = 10 litters)									
Mean (SD)	3.88 (0.23)		3.96 (0.20)		4.08 (0.30)		4.01 (0.23)			
% change ^b	–		2%		5%		3%			

Reference and study design	Results									
High (1.2)										
<i>Spermatogenic measures</i>										
van der Ven et al. (2009) Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Data quality:^e High (1.2)	Doses (mg/kg-d)									
		0	0.1	0.3	1	3	10	30	100	
	Epididymal sperm with separate heads (% of total)									
	Male, F1, PNW 11 (n = 4–5)**									
	Mean (SD)	4.2 (1.7)	3.8 (2.9)	7.5 (8.1)	2.2 (1.9)	4.4 (1.9)	4.1 (2.1)	5.0 (1.8)	0.8 (0.8)	
% change ^b	–	–10%	79%	–48%	5%	–2%	19%	–81%		
van der Ven et al. (2006) Rats, Wistar Gavage 28-d exposure starting on PNW 11 Data quality:^e High (1.3)	Doses (mg/kg-d)									
		0	0.3	1	3	10	30	100	200	
	Epididymal sperm with separate heads (% of total)									
	Male (n = 4–5)									
	Mean (SD)	5.3 (2.9)	3.8 (2.2)	7.4 (3.2)	4.7 (3.4)	5.1 (4.0)	6.8 (4.1)	3.5 (2.7)	5.1 (3.6)	
% change ^b	–	–28%	40%	–11%	–4%	28%	–34%	–4%		
<i>Reproductive organ weights</i>										
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	Doses (mg/kg-d)									
	F1, offspring^a	0		17		168		1,570		
	Male, F1, adult	0		11		115		1,142		
	F2, offspring^a	0		15		139		1,360		
	Relative epididymis weight (left and right) (mg/100 g BW)									
	Male, F1, PND 26 (n = 17–23)									
	Mean (SD)	85.9 (9.8)		86.7 (10.3)		89.3 (7.5)		89.9 (15.3)		
	% change ^b	–		1%		4%		5%		
	Male, F1 adult (n = 22–24)									
	Mean (SD)	223 (24)		232 (24)		210 (19)		234 (23)		
% change ^b	–		4%		–6%		5%			
Male, F2, PND 26 (n = 13–22)										

Reference and study design	Results									
Data quality:^c High (1.0)	Mean (SD)	90.7 (14.1)	87.2 (10.6)	87.3 (9.6)	96.2 (10.5)					
	% change ^b	–	–4%	–4%	6%					
	Relative testis weight (left and right) (mg/100 g BW)									
	Male, F1, PND 26 (n = 17–23)									
	Mean (SD)	0.57 (0.07)	0.61* (0.06)	0.62* (0.06)	0.63* (0.07)					
	% change ^b	–	9%	9%	12%					
	Male, F1 adult (n = 22–24)									
	Mean (SD)	0.60 (0.07)	0.61 (0.05)	0.58 (0.06)	0.59 (0.07)					
	% change ^b	–	2%	–4%	–1%					
	Male, F2, PND 26 (n = 13–22)									
	Mean (SD)	0.57 (0.01)	0.60 (0.06)	0.57 (0.09)	0.59 (0.05)					
	% change ^b	–	5%	0%	3%					
	Relative ventral prostate weight (mg/100 g BW)									
	Male, F1, PND 26 (n = 17–23)									
	Mean (SD)	46.4 (10.3)	47.1 (8.8)	48.2 (7.3)	44.5 (11.1)					
% change ^b	–	2%	4%	–4%						
Male, F1 adult (n = 22–24)										
Mean (SD)	137 (28)	135 (34)	131 (30)	135 (22)						
% change ^b	–	–1%	–4%	–1%						
Male, F2, PND 26 (n = 13–22)										
Mean (SD)	50.2 (9.3)	50.2 (10.7)	50.8 (9.6)	47.3 (15.8)						
% change ^b	–	0%	1%	–6%						
van der Ven et al. (2009) Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post	Doses (mg/kg-d)									
	Male, F1	0	0.1	0.3	1	3	10	30	100	
	Absolute epididymis weight (left and right) (g)									
	Male, F1, PNW 11 (n = 4–5)									
	Mean (SD)	0.95 (0.13)	0.88 (0.13)	0.95 (0.12)	1.00 (0.06)	0.90 (0.09)	0.85 (0.13)	0.98 (0.14)	0.82 (0.06)	
	% change ^b	–	–7%	0%	5%	–5%	–11%	3%	–14%	
	Absolute testis weight (left and right) (g)									
	Male, F1, PNW 11 (n = 4–5)**									
	Mean (SD)	3.01 (0.17)	2.91 (0.08)	3.07 (0.42)	3.18 (0.20)	2.88 (0.28)	2.82 (0.07)	2.97 (0.25)	2.60 (0.06)	
	% change ^b	–	–3%	2%	6%	–4%	–6%	–1%	–14%	

Reference and study design	Results								
weaning through PNW 11 Data quality: ^e High (1.2)	Absolute prostate weight (g)								
	Male, F1, PNW 11 (n = 4-5)**								
	Mean	0.66	0.73	0.57	0.73	0.57	0.58	0.67	0.42
	(SD)	(0.18)	(0.21)	(0.15)	(0.21)	(0.12)	(0.07)	(0.09)	(0.13)
	% change ^b	-	11%	-14%	11%	-14%	-12%	2%	-36%
WIL Research (2001) Rats, Crl:CD(SD)IGS BR Gavage 90 d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown Data quality: ^e High (1.0)	Absolute seminiferous vesicle weight (g)								
	Male, F1, PNW 11 (n = 4-5)								
	Mean	1.00	1.07	1.32	1.14	1.21	1.07	1.21	1.09
	(SD)	(0.40)	(0.22)	(0.23)	(0.29)	(0.09)	(0.29)	(0.25)	(0.27)
	% change ^b	-	7%	32%	14%	21%	7%	21%	9%
WIL Research (2001) Rats, Crl:CD(SD)IGS BR Gavage 90 d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown Data quality: ^e High (1.0)	Doses (mg/kg-d)								
	Male	0		100		300		1,000	
	Relative prostate weight (g/100 g BW)								
	Male (n = 9-10)								
	Mean	0.18 (0.03)		0.19 (0.03)		0.21 (0.04)		0.26 (0.05)	
	(SD)								
	% change ^b	-		3%		17%		42%	
	Relative testis weight (left) (g/100 g BW)								
	Male (n = 9-10)								
	Mean	0.30 (0.08)		0.31 (0.04)		0.31 (0.04)		0.32 (0.04)	
	(SD)								
	% change ^b	-		4%		2%		7%	
	Relative testis weight (right) (g/100 g BW)								
	Male (n = 9-10)								
	Mean	0.31 (0.07)		0.31 (0.04)		0.31 (0.04)		0.32 (0.05)	
	(SD)								
	% change ^b	-		0%		1%		6%	
	Relative cauda epididymis weight (left) (g/100 g BW)								
	Male (n = 9-10)								
Mean	0.05 (0.01)		0.06 (0.01)		0.06 (0.01)		0.06 (0.01)		
(SD)									
% change ^b	-		9%		6%		15%		
Relative cauda epididymis weight (right) (g/100 g BW)									
Male (n = 9-10)									
Mean	0.05 (0.01)		0.06 (0.01)		0.06 (0.01)		0.06 (0.01)		
(SD)									
% change ^b	-		6%		4%		17%		
Relative epididymis weight (left) (g/100 g BW)									
Male (n = 9-10)									
Mean	0.12 (0.02)		0.13 (0.01)		0.12 (0.02)		0.14 (0.01)		
(SD)									
% change ^b	-		8%		3%		13%		
Relative epididymis weight (right) (g/100 g BW)									

Reference and study design	Results				
	Male (n = 9–10)				
	Mean (SD)	0.12 (0.04)	0.13 (0.01)	0.13 (0.01)	0.14 (0.02)
	% change ^b	–	8%	3%	16%
Saegusa et al. (2009) Rats, Crj:CD(SD)IGS Diet	Doses (mg/kg-d)^d				
	Male, F1	0	14.8	146.3	1,505
	Relative epididymis weight (left and right) (g/100 g BW)				
	Male, F1, PND 20 (n = 10)				
	Mean (SD)	0.06 (0.02)	0.07 (0.01)	0.07 (0.01)	0.07 (0.01)
	% change ^b	–	8%	13%	8%
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.23 (0.02)	0.21* (0.01)	0.22 (0.02)	0.21 (0.01)
	% change ^b	–	–9%	–4%	–9%
	Relative testis weight (left and right) (g/100 g BW)				
	Male, F1, PND 20 (n = 10)				
	Mean (SD)	0.43 (0.04)	0.43 (0.03)	0.43 (0.05)	0.40 (0.03)
	% change ^b	–	0%	0%	–7%
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.77 (0.07)	0.73 (0.04)	0.78 (0.09)	0.74 (0.05)
	% change ^b	–	–5%	1%	–4%
	Relative dorsolateral prostate weight (mg/100 g BW)				
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.13 (0.03)	0.13 (0.01)	0.14 (0.03)	0.13 (0.02)
	% change ^b	–	0%	8%	0%
	Relative ventral prostate weight (mg/100 g BW)				
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.13 (0.02)	0.13 (0.04)	0.12 (0.03)	0.12 (0.01)
	% change ^b	–	0%	–8%	–8%
	Relative seminal vesicle weight (mg/100 g BW)				
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.27 (0.05)	0.26 (0.03)	0.26 (0.05)	0.26 (0.05)
	% change ^b	–	–4%	–4%	–4%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.

**Significant dose response trend as reported by study authors.

^aF1 and F2 offspring doses presented as mean maternal gestational and lactational F0 and F1 doses, respectively.

^bPercent change compared to control calculated as: (treated value – control value)/control value × 100.

^cExact number of animals examined per dose group was unclear in the published paper.

^dTWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PND 1–9, and PND 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = $(8.1 \text{ mg/kg-day} \times 11 \text{ days}) + (14.3 \text{ mg/kg-day} \times 10 \text{ days}) + (21.3 \text{ mg/kg-day} \times 12 \text{ days}) / 33 \text{ days} = 14.8 \text{ mg/kg-day}$.

^eBased on OPPT data evaluation criteria

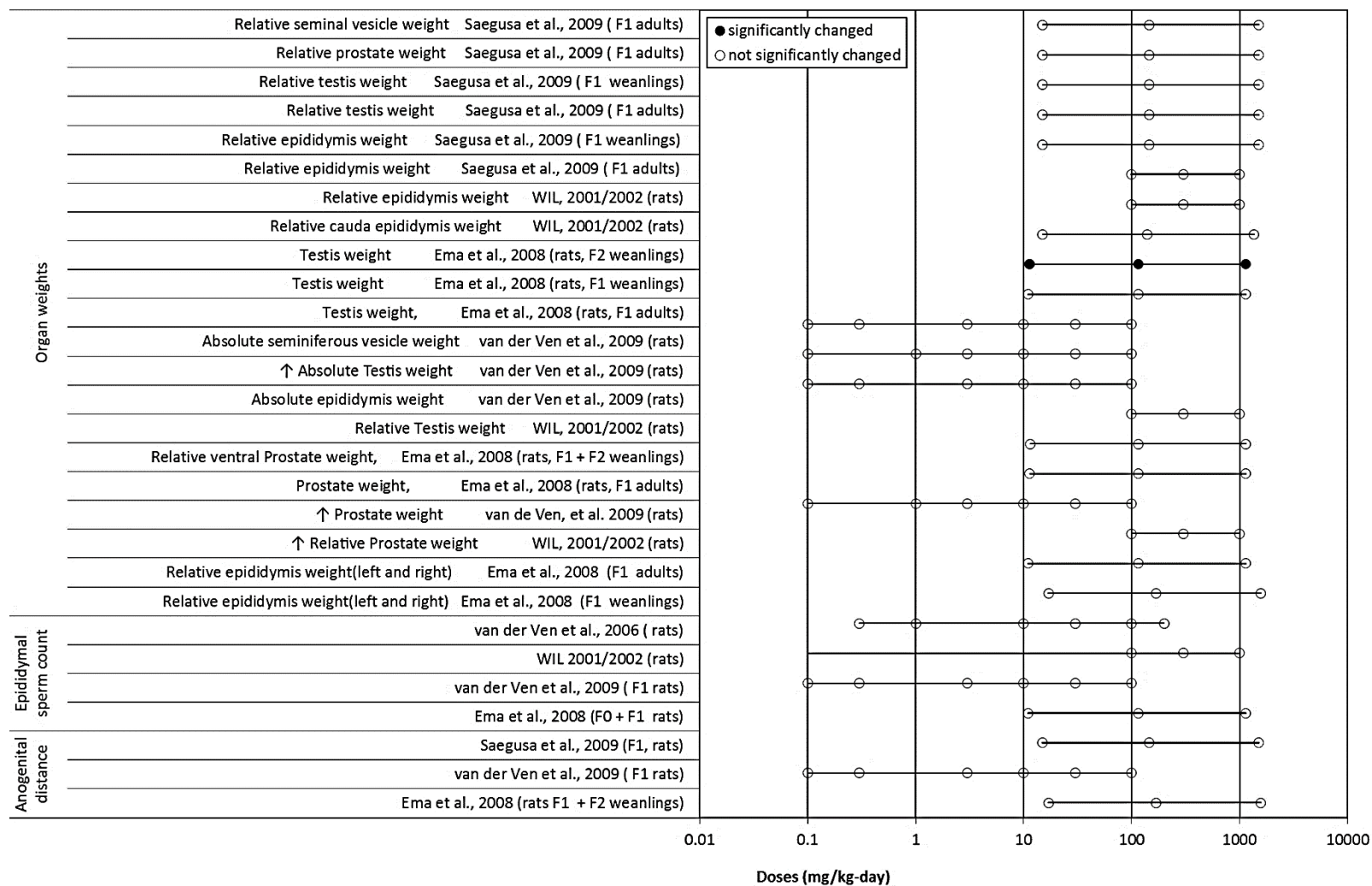


Figure 1-5. Exposure response array of male reproductive system effects following oral exposure. All studies scored a High in data quality evaluation.

1.3.2.3 Mechanistic Evidence

See Section 1.3.1.3 in the Female Reproductive Effects section above (Mechanistic Evidence).

1.4 Developmental Effects

1.4.2 Human Evidence

Epidemiology studies investigating potential thyroid, male reproductive, and nervous system effects of HBCD following developmental exposure were identified and are discussed in their respective organ/system-specific hazard sections (Sections 1.1.1, 1.3.2.1, and 1.5.1, respectively).

1.4.3 Animal Evidence

Evidence to inform organ-system specific effects of HBCD in animals following developmental exposure are discussed in the individual hazard sections. The current section is limited to discussion of developmental specific effects, including offspring survival, pup body weight, developmental markers, and bone measures.

HBCD-induced developmental effects, including offspring survival, body weight, and developmental markers, were evaluated in five studies in rats ([Hachisuka et al., 2010](#); [Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#)) and mice ([Maranghi et al., 2013](#)), with exposure durations ranging from 28 days in juvenile mice to continuous exposure of rats over two generations. A summary of developmental effects associated with HBCD exposure is presented in Table 1-7 and Figure 1-6. Effect categories with stronger evidence are presented first, with individual studies ordered by study duration and then species. For each endpoint, age at outcome measurement is indicated.

Effects on offspring survival and pup body weight were evaluated in three rat studies ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#)) and juvenile body weight was reported in a single mouse study ([Maranghi et al., 2013](#)). Two rat studies that utilized similar dose ranges (approximately 10–1,500 mg/kg-day) reported statistically significant effects in the high-dose group ([Saegusa et al., 2009](#); [Ema et al., 2008](#)). [Ema et al. \(2008\)](#) reported decreases in pup body weight ranging from 20 to 25% for male and female F2 rat pups on PNDs 7, 14, and 21. Offspring survival on PNDs 4 and 21 (21 and 42%, respectively) in this dose group was also decreased ([Ema et al., 2008](#)). Decreases in pup weight in F1 animals were smaller (<10%), did not show a consistent pattern of effect, and were not associated with decreased viability ([Saegusa et al., 2009](#); [Ema et al., 2008](#)). The remaining studies indicate a potential for HBCD to decrease body weight ([Maranghi et al., 2013](#); [van der Ven et al., 2009](#)) but not viability ([van der Ven et al., 2009](#)) at lower doses (up to 199 mg/kg-day). [van der Ven et al. \(2009\)](#) reported significant dose-dependent trends in decreased body weight in male and female rat pups. Similarly, [Maranghi et al. \(2013\)](#) reported a 14% body weight decrease in juvenile female mice exposed for 28 days, although this effect was not statistically significant. Use of a single-dose study design did not allow for evaluation of dose-response in this study.

Treatment-related effects on several developmental landmarks were evaluated in F1 and F2 offspring in the two-generation reproductive toxicity study ([Ema et al., 2008](#)). In F1 pups, eye opening on PND 14 was significantly increased in both sexes in the mid-dose group, but not the

high-dose group (approximately 170 and 1,500 mg/kg-day, respectively). In contrast, F2 offspring exhibited statistically significant dose-related decreases in eye opening on PND 14 in both the mid- (females only) and high-dose groups (males and females). Other developmental landmarks (i.e., pinna unfolding, and incisor eruption) were not affected ([Ema et al., 2008](#)).

Measures of bone development were also evaluated in rats treated continuously from gestation through adulthood at doses up to 100 mg/kg-day ([van der Ven et al., 2009](#)). Trabecular bone mineral density in females was decreased by 20%. The study authors reported dose-related decreases in several other tibia related endpoints; however, the magnitude of these effects was small and inconsistent across dose group and sex, making it difficult to interpret the biological significance of these findings.

Table 1-7. Evidence pertaining to developmental effects in animals following exposure to HBCD

Reference and study design	Results				
<i>Fetal and early postnatal survival</i>					
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Data quality: ^f High (1.0)	Doses (mg/kg-d)				
	F1 offspring^a	0	17	168	1,570
	F2 offspring^a	0	15	139	1,360
	Viability index (%)				
	F1, PND 0 (n = 18–24 litters)				
	Mean (SD)	99.6 (1.9)	97.5 (8.5)	98.8 (2.8)	99.2 (2.5)
	% of control ^b	–	–2%	–1%	0%
	F1, PND 4 (n = 18–24 litters)				
	Mean (SD)	95.6 (8.6)	98.7 (2.8)	98.7 (4.4)	95.8 (10.3)
	% of control ^b	–	3%	3%	0%
	F1, PND 21 (n = 18–24 litters)				
	Mean (SD)	93.2 (17.3)	99.4 (2.7)	98.1 (4.6)	93.8 (23.6)
	% of control ^b	–	7%	5%	1%
F2, PND 0 (n = 20–23 litters)					
Mean (SD)	98.6 (5.3)	97.7 (4.9)	96.0 (9.5)	97.8 (5.1)	
% of control ^b	–	–1%	–3%	–1%	
F2, PND 4 (pre-culling) (n = 20–23 litters)					
Mean (SD)	86.9 (24.8)	87.3 (21.1)	92.1 (12.8)	68.4* (33.5)	
% of control ^b	–	0%	6%	–21%	
F2, PND 21 (n = 20–22 litters)					
Mean (SD)	85.0 (22.0)	89.6 (13.9)	71.3 (26.9)	49.7* (41.1)	
% of control ^b	–	5%	–16%	–42%	
Saegusa et al. (2009) Rats, Crj:CD(SD)IGS Diet	Doses (mg/kg-d)^c				
		0	15	146	1,505
	Number of live pups				
	Female, F0 (n = 10 litters)				

Reference and study design	Results				
F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11	Mean (SD)	13.0 (1.8)	13.0 (1.6)	11.6 (1.6)	12.9 (1.4)
	% of control ^b	–	0%	–11%	–1%
Data quality:^f High (1.2)					
<i>Body weight</i>					
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation	Doses (mg/kg-d)				
	F1 offspring^a	0	17	168	1,570
	F2 offspring^a	0	15	139	1,360
F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	Pup weight (g)				
	Male, F1, PND 0 (n = 18–24 litters)				
	Mean (SD)	6.8 (0.5)	6.9 (0.6)	7.2 (0.7)	6.8 (0.6)
	% of control ^b	–	1%	6%	0%
	Male, F1, PND 4 (n = 18–24 litters)				
	Mean (SD)	10.2 (1.7)	10.7 (1.8)	10.8 (1.6)	9.5 (1.8)
	% of control ^b	–	5%	6%	–7%
	Male, F1, PND 7 (n = 17–24 litters)				
	Mean (SD)	16.4 (3.1)	17.5 (2.4)	16.9 (2.2)	15.6 (2.0)
	% of control ^b	–	7%	3%	–5%
	Male, F1, PND 14 (n = 17–23 litters)				
	Mean (SD)	36.1 (4.8)	36.3 (3.6)	36.1 (3.9)	33.5 (2.6)
	% of control ^b	–	1%	0%	–7%
	Male, F1, PND 21 (n = 17–23 litters)				
	Mean (SD)	61.1 (7.1)	62.3 (6.5)	61.9 (6.5)	55.4* (4.0)
	% of control ^b	–	2%	1%	–9%
	Female, F1, PND 0 (n = 18–23 litters)				
	Mean (SD)	6.3 (0.5)	6.6 (0.7)	6.8* (0.6)	6.5 (0.7)
	% of control ^b	–	5%	8%	3%
	Female, F1, PND 4 (n = 18–23 litters)				
	Mean (SD)	9.6 (1.4)	10.3 (1.8)	10.4 (1.5)	9.2 (1.6)
	% of control ^b	–	7%	8%	–4%
	Female, F1, PND 7 (n = 17–23 litters)				
	Mean (SD)	15.4 (2.8)	17.0 (2.5)	16.9 (2.3)	15.1 (1.6)
	% of control ^b	–	10%	10%	–2%
	Female, F1, PND 14 (n = 17–23 litters)				
	Mean (SD)	33.5 (5.3)	35.5 (3.6)	35.7 (3.6)	32.6 (3.0)
	% of control ^b	–	6%	7%	–3%
	Female, F1, PND 21 (n = 17–23 litters)				
Data quality:^f High (1.0)					

Reference and study design	Results								
	Mean (SD)	56.5 (8.0)	59.9 (6.4)	60.5 (5.9)	53.2 (4.7)				
	% of control ^b	–	6%	7%	–6%				
	Male, F2, PND 0 (n = 20–23 litters)								
	Mean (SD)	6.8 (0.8)	6.7 (0.7)	7.1 (0.6)	6.6 (0.6)				
	% of control ^b	–	–1%	4%	–3%				
	Male, F2, PND 4 (n = 19–22 litters)								
	Mean (SD)	9.1 (2.3)	9.3 (1.3)	9.0 (1.8)	8.0 (1.3)				
	% of control ^b	–	2%	–1%	–12%				
	Male, F2, PND 7 (n = 17–22 litters)								
	Mean (SD)	14.7 (3.9)	15.4 (2.8)	14.3 (3.6)	11.5* (2.9)				
	% of control ^b	–	5%	–3%	–22%				
	Male, F2, PND 14 (n = 14–22 litters)								
	Mean (SD)	31.4 (8.0)	33.8 (5.0)	31.0 (7.2)	24.2* (6.6)				
	% of control ^b	–	8%	–1%	–23%				
	Male, F2, PND 21 (n = 13–22 litters)								
	Mean (SD)	53.0 (12.6)	56.2 (6.7)	54.1 (10.1)	42.6* (8.3)				
	% of control ^b	–	6%	2%	–20%				
	Female, F2, PND 0 (n = 20–23 litters)								
	Mean (SD)	6.5 (0.8)	6.3 (0.6)	6.7 (0.6)	6.2 (0.6)				
	% of control ^b	–	–3%	3%	–5%				
	Female, F2, PND 4 (n = 20–22 litters)								
	Mean (SD)	8.9 (2.3)	8.5 (1.3)	8.8 (1.8)	7.3* (1.3)				
	% of control ^b	–	–5%	–1%	–22%				
	Female, F2, PND 7 (n = 17–22 litters)								
Mean (SD)	14.3 (3.5)	14.2 (2.8)	13.5 (3.9)	10.7* (2.6)					
% of control ^b	–	–1%	–6%	–25%					
Female, F2, PND 14 (n = 13–22 litters)									
Mean (SD)	31.2 (6.5)	31.3 (5.1)	29.3 (7.3)	23.9* (5.9)					
% of control ^b	–	0%	–6%	–23%					
Female, F2, PND 21 (n = 13–22 litters)									
Mean (SD)	52.0 (10.0)	52.8 (6.6)	51.2 (10.8)	41.6* (8.4)					
% of control ^b	–	2%	–2%	–20%					
van der Ven et al. (2009) Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two	Doses (mg/kg-d)								
		0	0.1	0.3	1	3	10	30	100
	Pup weight (g)								
	Male, F1, PND 4 (n ≥ 14) ^{d **}								
	Mean (SD)	10.0 (1.3)	10.2 (0.7)	9.8 (1.2)	10.8 (1.9)	10.2 (1.7)	10.8 (1.4)	11.0 (1.3)	9.5 (0.9)
% of control ^b	–	2%	–2%	8%	2%	8%	10%	–5%	
Male, F1, PND 7 (n ≥ 14) ^d									

Reference and study design	Results									
estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Data quality:^f High (1.2)	Mean (SD)	13.4 (2.2)	13.6 (1.6)	12.7 (2.0)	14.7 (4.1)	13.1 (3.0)	13.9 (2.7)	14.6 (1.7)	12.6 (1.0)	
	% of control ^b	–	1%	–5%	10%	–2%	4%	9%	–6%	
	Male, F1, PND 14 (n ≥ 14)^{d **}									
	Mean (SD)	22.3 (6.4)	24.2 (5.0)	22.0 (4.0)	33.3 (8.6)	24.1 (7.7)	24.6 (6.5)	22.5 (3.2)	20.5 (2.2)	
	% of control ^b	–	9%	–1%	49%	8%	10%	1%	–8%	
	Male, F1, PND 21 (n ≥ 14)^{d **}									
	Mean (SD)	39.3 (7.5)	41.8 (8.9)	35.1 (5.2)	55.7 (14.4)	39.1 (12.0)	39.5 (10.0)	35.6 (6.2)	32.2 (3.0)	
	% of control ^b	–	6%	–11%	42%	–1%	1%	–9%	–8%	
	Female, F1, PND 4 (n ≥ 14)^{d **}									
	Mean (SD)	9.5 (1.5)	9.7 (0.8)	9.4 (1.1)	10.6 (2.7)	9.4 (1.5)	10.8 (1.1)	10.7 (1.2)	8.9 (0.9)	
	% of control ^b	–	2%	–1%	12%	–1%	14%	13%	–6%	
	Female, F1, PND 7 (n ≥ 14)^{d **}									
	Mean (SD)	12.9 (2.6)	12.8 (1.4)	12.4 (2.1)	14.2 (5.1)	12.5 (2.7)	14.4 (2.2)	14.1 (1.7)	11.9 (1.3)	
	% of control ^b	–	–1%	–4%	10%	–3%	12%	9%	–8%	
	Female, F1, PND 14 (n ≥ 14)^{d **}									
Mean (SD)	23.6 (5.3)	23.1 (2.7)	21.0 (3.8)	31.1 (7.9)	22.4 (6.0)	24.7 (5.8)	22.5 (4.4)	20.0 (2.9)		
% of control ^b	–	–2%	–11%	32%	–5%	5%	–5%	–15%		
Female, F1, PND 21 (n ≥ 14)^{d **}										
Mean (SD)	40.3 (8.6)	40.1 (5.9)	34.1 (5.4)	50.4 (11.9)	37.0 (10.3)	40.0 (9.5)	37.5 (5.9)	32.3 (3.9)		
% of control ^b	–	0%	–15%	25%	–8%	–1%	–7%	–20%		
Saegusa et al. (2009) Rats, Crj:CD(SD)IGS Diet	Doses (mg/kg-d)^c									
	0	15	146	1,505						
	Pup weight (g)									
F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11 ^e Data quality:^f High (1.2)	Male, F1, PND 1 (n = 10 litters)									
	Mean (SD)	7.11 (0.66)		7.22 (0.56)		7.65 (0.95)		7.15 (0.80)		
	% of control ^b	–	2%		8%		1%			
	Male, F1, PND 20 (n = 10)									
	Mean (SD)	54.3 (3.5)		51.2 (7.3)		56.7 (4.1)		54.0 (3.3)		
	% of control ^b	–	–6%		4%		–1%			
	Male, F1, at puberty onset ~PND 40 (n = 12–14)									
	Mean (SD)	204.3 (15.7)		198.3 (20.4)		203.2 (15)		195.8 (10.1)		
	% of control ^b	–	–3%		–1%		–4%			
	Male, F1, PNW 11 (n = 10)									
Mean (SD)	454.3 (25.4)		456.9 (24.8)		450.8 (33.4)		435.1 (24.6)			
% of control ^b	–	1%		–1%		–4%				

Reference and study design	Results				
	Female, F1, PND 1 (n = 10 litters) ^c				
	Mean (SD)	6.53 (0.59)	6.84 (0.50)	7.28 (0.75)	6.84 (0.81)
	% of control ^b	–	5%	11%	5%
	Female, F1, PND 20 (n = 10)				
	Mean (SD)	50.3 (3.4)	50.0 (6.0)	53.7 (5.5)	51.3 (2.9)
	% of control ^b	–	–1%	7%	2%
	Female, F1, at puberty onset ~PND 35 (n = 12–14)				
	Mean (SD)	130.8 (11.7)	133.8 (10.8)	129.2 (13.5)	118.6* (11.7)
	% of control ^b	–	2%	–1%	–9%
	Female, F1, PNW 11 (n = 10)				
	Mean (SD)	286.2 (25.2)	293.4 (21.5)	289.2 (24.4)	270.7 (19.6)
	% of control ^b	–	3%	1%	–5%
Maranghi et al. (2013) Mice, BALB/c Females only Diet 28-d exposure starting on PND 26 Data quality: ^f High (1.2)	Doses (mg/kg-d)				
		0		199	
	Body weight gain (g)				
	Female, PND 54 (n = 10–15)				
	Mean (SD)	5.80 (0.74)		5.00 (1.16)	
% of control ^b	–		–14%		
<i>Developmental markers</i>					
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Data quality: ^f High (1.0)	Doses (mg/kg-d)				
	F1 offspring^a	0	17	168	1,570
	F2 offspring^a	0	15	139	1,360
	Eye opening (%)				
	Male, F1, PND 14 (n = 17–23 litters)				
	Mean (SD)	48.2 (41.5)	56.7 (37.9)	77.1* (36.3)	45.8 (34.6)
	% of control ^b	–	18%	60%	–5%
	Female, F1, PND 14 (n = 17–23 litters)				
	Mean (SD)	49.3 (37.8)	66.7 (41.3)	82.9* (33.5)	54.9 (41.4)
	% of control ^b	–	35%	68%	11%
	Male, F2, PND 14 (n = 14–22 litters)				
Mean (SD)	72.7 (40.0)	62.5 (40.6)	47.2 (44.8)	33.9* (34.7)	
% of control ^b	–	–14%	–35%	–53%	
Female, F2, PND 14 (n = 13–21 litters)					
Mean (SD)	82.9 (26.8)	72.7 (37.7)	53.8* (40.3)	48.1* (42.0)	
% of control ^b	–	–12%	–35%	–42%	
No exposure-related changes were found in incisor eruption (PND 11) or pinna unfolding (PND 3).					

Reference and study design	Results									
<i>Bone measures</i>										
van der Ven et al. (2009) Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Data quality:^f High (1.2)	Doses (mg/kg-d)									
		0	0.1	0.3	1	3	10	30	100	
	Trabecular bone mineral density, tibia (mg/cm³)									
	Male, F1, PNW 11 (n = 4–5)									
	Mean	145	143	154	167	134	146	156	167	
	(SD)	(25)	(20)	(23)	(16)	(36)	(25)	(20)	(11)	
	% of control ^b	–	–1%	6%	15%	–8%	1%	8%	15%	
	Female, F1, PNW 11 (n = 5)**									
	Mean	294	268	253	231	245	227	200	234	
	(SD)	(19)	(27)	(30)	(35)	(31)	(28)	(31)	(29)	
% of control ^b	–	–9%	–14%	–21%	–17%	–23%	–32%	–20%		

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.

**Significant dose response trend as reported by study authors.

^aF1 and F2 offspring doses presented as mean maternal gestational and lactational F0 and F1 doses, respectively.

^bPercent change compared to control calculated as: $(\text{treated value} - \text{control value}) / \text{control value} \times 100$.

^cTWA doses for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PNDs 1–9, and PNDs 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: $100 \text{ ppm} = (8.1 \text{ mg/kg-day} \times 11 \text{ days}) + (14.3 \text{ mg/kg-day} \times 10 \text{ days}) + (21.3 \text{ mg/kg-day} \times 12 \text{ days}) / 33 \text{ days} = 14.8 \text{ mg/kg-day}$.

^dExact number of animals examined per dose group was unclear based on the published paper.

^e[Saegusa et al. \(2009\)](#) and [Hachisuka et al. \(2010\)](#) appear to be two publications of the same animal cohort; the TWA doses calculated for [Saegusa et al. \(2009\)](#) were applied to [Hachisuka et al. \(2010\)](#).

^fBased on OPPT data evaluation criteria

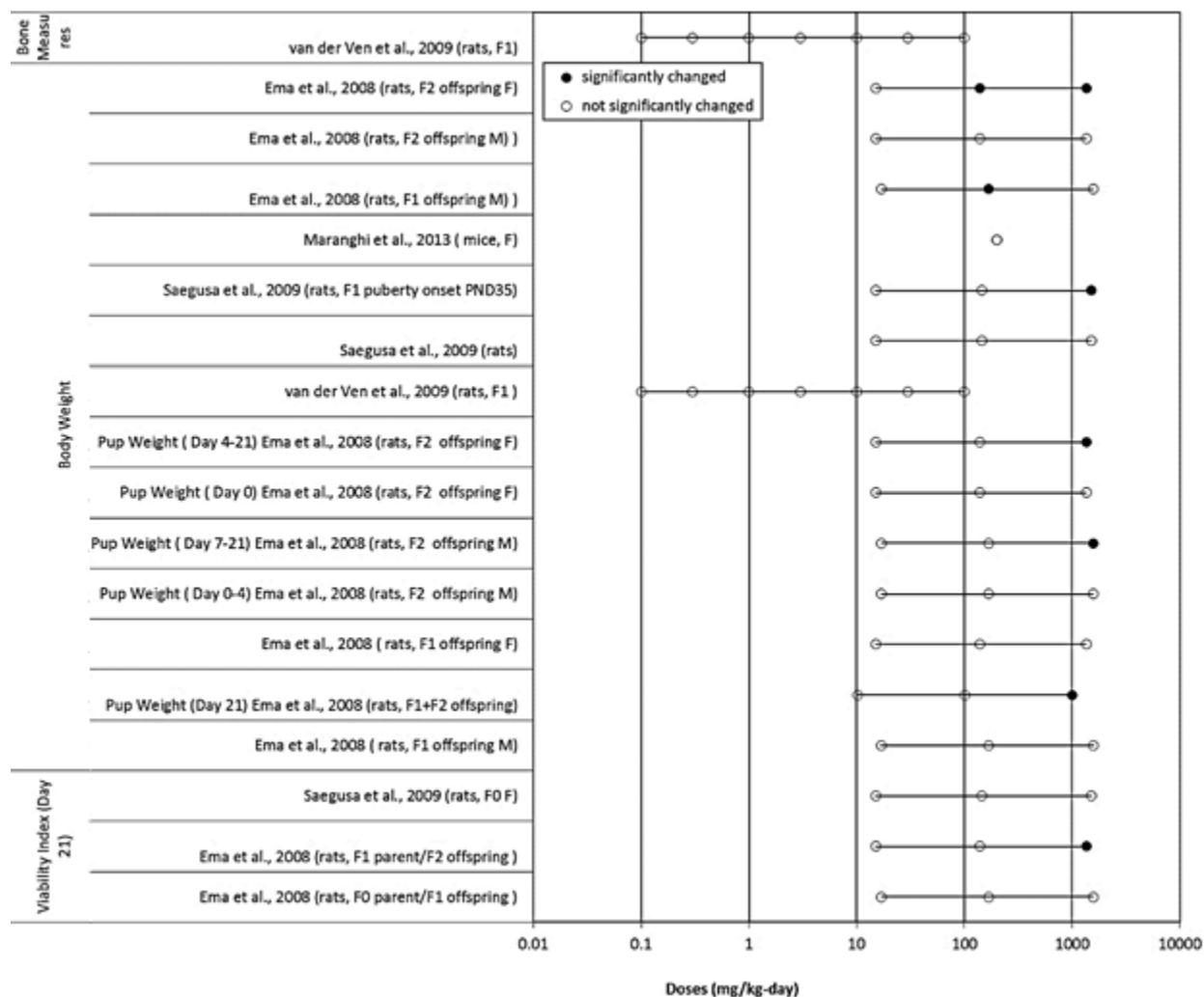


Figure 1-6. Exposure response array of developmental effects following oral exposure. All studies scored High in data quality evaluation.

1.4.4 Mechanistic Evidence

Studies directly investigating mechanistic evidence to inform potential developmental effects of HBCD are limited to a few studies in zebrafish (Wu et al., 2013; Du et al., 2012; Deng et al., 2009; Hu et al., 2009a), which focus on identifying molecular targets that drive HBCD-mediated perturbation of normal embryonic development. In general, HBCD exposure was associated with increased ROS generation and induction of apoptotic cell pathways resulting in malformations and reduced viability in zebrafish (Du et al., 2012; Deng et al., 2009; Hu et al., 2009a). In the absence of overt teratogenic effects, HBCD exposure was found to affect cardiac function and development, resulting in increased heart rate, arrhythmia, cardiac hypertrophy, and increased collagen deposition; these effects were associated with changes in expression of genes associated with calcium transport and cardiomyocyte conduction (Wu et al., 2016; Wu et al., 2013). In rat cardiomyocytes (H9C2), HBCD treatment altered Ca²⁺ signaling through changes in expression of several genes (Ryr2, Serca2a, and Ncx1) involved in Ca²⁺ regulation (Wu et al., 2016).

Although no studies were identified that directly investigated the potential for HBCD-driven thyroid hormone imbalances to induce developmental effects, in vivo studies provide evidence of

an association between HBCD exposure and disrupted homeostasis of thyroid hormones (see Section 1.2.1), which are critical regulators of growth and development. In humans, umbilical T4 concentrations are positively correlated with body weight and length at birth ([Shields et al., 2011](#)) and cases of intrauterine growth restriction and small-for-gestational-age fetuses are associated with reduced thyroid hormone levels in both human populations and experimental animals ([Forhead and Fowden, 2014](#); [Pererira and Procianoy, 2003](#)). Thyroidectomy in fetal sheep reduces total body and organ weights and affects bone development, including delayed maturation and altered bone strength and mineral density ([Forhead and Fowden, 2014](#); [Lanham et al., 2011](#)); these effects were ameliorated by T4 replacement ([Forhead and Fowden, 2014](#)). Furthermore, human congenital hypothyroidism is also associated with neurological and skeletal abnormalities, even when birth weight is unaffected ([Patel et al., 2011](#); [Shields et al., 2011](#)). Based on the broader developmental literature, it is plausible that developmental effects observed following HBCD exposure could be a consequence of HBCD-induced changes in thyroid homeostasis; however, HBCD-specific data to support this relationship are not available.

1.5 Nervous System Effects

1.5.2 Human Evidence

Epidemiology studies have been conducted in children participating in birth cohort studies in the Netherlands ([Roze et al., 2009](#)) and in adolescents in a cross-sectional general population study in areas around industrial sites in Belgium ([Kiciński et al., 2012](#)) (Table 1-8). In a study of children ages 5–6 years (n = 62), maternal HBCD levels measured at week 35 of pregnancy were associated with increased scores for three neuropsychological domains (coordination, total intelligence, and verbal intelligence) after adjusting for maternal education, home environment (Home Observation for Measurement of the Environment [HOME] score), and sex ([Roze et al., 2009](#)). The authors stated that no associations were observed between HBCD and the other tested domains (visual perception, visuomotor integration, inhibitory control, attention, behavior, and attention deficit/hyperactivity disorder), but did not report effect estimates for these measures. [Kiciński et al. \(2012\)](#) did not observe associations between HBCD levels and six neurobehavioral measures assessing attention, visual scanning and information processing, working memory, and motor function in a study in adolescents (ages 13–17; n = 515); this analysis was based on HBCD exposure dichotomized at concentrations above and below the LOQ (30 ng/L) because 75% of values were less than the LOQ. Interpretation of the results of these studies is limited by poor reporting of results and small sample size in the study by [Roze et al. \(2009\)](#), and by low HBCD detection rates (<25%) in the study population and measure of HBCD in adolescents that does not represent a relevant time window of exposure for neurodevelopmental outcomes in the case of [Kiciński et al. \(2012\)](#). Thus, the available evidence for an association between HBCD exposure and nervous system effects in humans is insufficient for drawing conclusions.

1.5.3 Animal Evidence

The potential for HBCD to affect the nervous system has been examined in 10 studies in rats ([Genskow et al., 2015](#); [Miller-Rhodes et al., 2014](#); [Lilienthal et al., 2009](#); [Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#); [Eriksson et al., 2006](#); [van der Ven et al., 2006](#); [WIL](#)

[Research, 2001, 1997](#)) with exposures ranging from a single gavage dose on PND 10 to continuous exposure across two generations.

Discussion of nervous system-related effects is organized by the timing of exposure (i.e., developmental and adult) due to the sensitivity of the developing nervous system to the effect of chemicals. A summary of the evidence pertaining to nervous system effects in experimental animals is presented in Table 1-9 and Figure 1-7. Individual studies are ordered by study duration and then species. If not otherwise indicated measurements were made in adults.

1.5.3.1 Developmental Exposure

Neurodevelopmental Milestones

Neurodevelopmental milestones were evaluated in two rat studies ([Miller-Rhodes et al., 2014](#); [Ema et al., 2008](#)). Gestational exposure to HBCD heightened tail pinch responses in pooled male and female rat pups (PNDs 1–21; 3–30 mg/kg-day) and reduced forelimb grip strength in juvenile male, but not female, rats (PND 26; 10 and 30 mg/kg-day) ([Miller-Rhodes et al., 2014](#)). Development of sensorimotor reflexes was affected in rats exposed to approximately 1,300 mg/kg-day in a two-generation reproductive toxicity study; however, effects were not consistent across generations, sex, or the reflex evaluated ([Ema et al., 2008](#)) and were not observed in a separate study ([Miller-Rhodes et al., 2014](#)). Differences in the experimental design (i.e., multigenerational versus developmental) and outcome recording (i.e., righting latency versus age at which $\geq 85\%$ of pups completed the behavior within 1 minute) may have contributed to differences in the surface righting reflex responses reported by these research groups. Furthermore, in the study by [Ema et al. \(2008\)](#), statistically significant effects on righting reflexes were only observed in exposure groups that also exhibited signs of overt toxicity (e.g., decreased body weight gain and pup survival); thus, changes in sensorimotor reflexes may be due to general toxicity rather than an organ system-specific effect.

Executive Function and Locomotor Activity

The effects of HBCD exposure on executive function (e.g., learning, memory, attention) were evaluated in three studies in rats ([Miller-Rhodes et al., 2014](#); [Ema et al., 2008](#)) and mice ([Eriksson et al., 2006](#)). [Miller-Rhodes et al. \(2014\)](#) evaluated performance on two operant tasks designed to measure sustained attention, response inhibition, and persistence in adult (11–14 months) and aging rats (19–21 months) that were exposed to HBCD in utero. The go/no-go task evaluated effects on sustained attention and response inhibition by requiring animals to discriminate between distinct visual cues that indicate whether a trial is reinforced for pressing the lever (i.e., go trial) or for abstaining from lever pressing (i.e., no-go trial). Combined responses from male and female offspring from the low-dose group (3 mg/kg-day) showed a statistically significant decrease in the number of correct lever presses and an increase in response latency; however, no effect was observed in the two higher dose groups. No treatment-related effects were observed in the random ratio task, which evaluated persistence behaviors by providing animals with intermittent reinforcement (i.e., food pellet reward) for lever pressing. Although these tests are sensitive indicators of altered cognitive function, the results are difficult to interpret as data were pooled across age cohorts. Furthermore, some aging animals in the 3 mg/kg-day group developed unexplained loss of hindlimb control that was not observed in controls or higher dose groups. To minimize the potential effects on these behavioral outcomes, litters containing animals that developed serious health complications were excluded from

analysis ([Miller-Rhodes et al., 2014](#)); however, it is possible that animals with less severe muscular degeneration were included.

Two studies evaluated learning ability using swim maze tests. A statistically significant increase in trial time on a Morris swim maze was observed in young adult (3-month-old) male mice exposed once to 13.5 mg/kg on PND 10; however, swim speed and visual acuity were not measured as possible confounders ([Eriksson et al., 2006](#)). In contrast, a statistically significant decrease in trial times on a multiple T-maze was reported on a single day of testing in juvenile F1 male rats (PNW 6) exposed to approximately 100–1,300 mg/kg-day ([Ema et al., 2008](#)). Females showed a similar pattern of behavior across multiple testing days, but changes were not statistically significant and the data showed high standard errors (SEs). Differences in the test species, exposure, and testing methods may have contributed to the different results of the two swim maze studies and complicates interpretation of these findings.

Three studies measured effects of early-life exposure on locomotor activity in rats ([Miller-Rhodes et al., 2014](#); [Ema et al., 2008](#)) and mice ([Eriksson et al., 2006](#)). [Eriksson et al. \(2006\)](#) evaluated effects in young adult (3-month-old) mice that were administered a single dose on PND 10, which corresponds with a period of rapid growth and maturation for motor and sensory neural networks in mice. Controls and mice exposed to 0.9 mg/kg showed a normal activity pattern, characterized by high initial activity that steadily decreased over the course of the 60-minute test period. The 13.5 mg/kg group, however, exhibited a moderate activity level that remained steady (i.e., significantly lower versus control activity at the beginning and significantly higher versus controls at the end of the test), suggesting failure to habituate to the novel environment of the testing arena. Similar testing methods were employed to evaluate locomotor activity in juvenile ([Ema et al., 2008](#)), young adult, and aging rats ([Miller-Rhodes et al., 2014](#)). Although both of these studies utilized longer exposure durations and higher doses, they found no effects on spontaneous locomotor activity ([Miller-Rhodes et al., 2014](#); [Ema et al., 2008](#)).

Other Neurological Effects

Effects on auditory function and dopamine-dependent movement behavior were evaluated in a single rat study that exposed animals continuously throughout gestation, lactation, and into adulthood ([Lilienthal et al., 2009](#)). Brainstem evoked auditory potentials (BAEPs) were measured to evaluate effects on auditory function. Study authors reported that males, but not females, showed a small dose-related trend towards increased thresholds and signal latency, suggesting reduced hearing sensitivity. In the same study, dopamine system effects were evaluated by measuring cataleptic movement latencies. atalepsy is a condition characterized by muscle rigidity and waxy flexibility (i.e., subject tends to remain in a fixed position, but the posture/limb position can be altered). A cataleptic state was induced by haloperidol, a drug that blocks dopamine receptors. Animals were then placed in fixed postures and movement latency was recorded. Statistically significant dose-dependent decreases in movement latency were reported in the catalepsy tests for both sexes, although effects were more pronounced in females. These results suggest that HBCD increases dopamine signaling. It was unclear, however, whether animals were given a recovery period between certain postures in the catalepsy tests, which may have stressed the animals and affected the results. In the BAEP test, the average increase in auditory threshold observed at the highest dose was 9 dB. Although BAEP is a

sensitive measure of auditory function, the changes observed in this study were below those generally considered to be biologically significant (10–15 dB).

Three studies evaluated brain weight changes in rats ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#)). Absolute brain weights showed a statistically significant reduction in F1 adults and both F1 and F2 weanlings in the high-dose group (approximately 1,300 mg/kg-day) ([Ema et al., 2008](#)); these animals also exhibited signs of overt toxicity, including decreased viability and pup weight (Section 1.2.4). [van der Ven et al. \(2009\)](#) also reported a significant trend for absolute brain weights in male rats at the end of a one-generation exposure, with most groups showing an increase relative to controls; brain weight changes were not observed in females. No statistically significant change in relative brain weight was observed in gestationally and lactationally exposed rats ([Saegusa et al., 2009](#)); however, relative brain weight changes are considered to be less informative of nervous system effects. Notably, brain weight changes are considered to be a relatively insensitive measure of neurotoxicity and, with the exception of the F2 high dose animals in [Ema et al. \(2008\)](#), the statistically significant effects were below the level that is considered to be biologically significant.

1.5.3.2 Adult Exposure

The four studies that evaluated neurotoxicity endpoints in adult animals did not provide evidence that HBCD exposure affects the nervous system at this life stage ([Genskow et al., 2015](#); [van der Ven et al., 2006](#); [WIL Research, 2001, 1997](#)). No gross changes in striatal levels of dopamine or its metabolites were observed in adult male mice exposed to 25 mg/kg-day HBCD for 30 days ([Genskow et al., 2015](#)). Similarly, no effects on other neurological measures, including a functional observational battery (FOB), locomotor activity, brain weight, or gross pathology were observed in adult rats exposed to up to 1,000 mg/kg-day HBCD for 90 ([WIL Research, 2001](#)) or 28 days ([van der Ven et al., 2006](#); [WIL Research, 1997](#)).

Table 1-8. Evidence pertaining to nervous system effects in humans

Reference and study design	Results	
<i>Studies in infants and children, neurodevelopment</i>		
Roze et al. (2009) (the Netherlands, COMPARE cohort, 2001–2002 at baseline) Population: Birth cohort, 90 singleton, term births, 62 of 69 (90%) mother-child pairs randomly selected from the cohort for HBCD measures in serum; children ages 5–6 years at follow-up Exposure measures: Prenatal exposure, maternal serum at 35 th week of pregnancy; 1,2,5,6,9,10-HBCD (HBCD) detected in all samples; LOD 0.8 pg/g serum	Correlations between lipid-adjusted HBCD and outcome measure adjusted for socioeconomic status (maternal education), HOME score, and sex	
	<i>Neuropsychological measure</i>	<i>Correlation coefficient</i>
	Coordination	0.290 ($p < 0.05$)
	Total intelligence	0.393 ($p < 0.05$)
	Verbal intelligence	0.479 ($p < 0.01$)

<p>Median 0.8 (range: 0.3–7.5) ng/g lipids</p> <p>Effect measures: Neuropsychological tests (references for procedure provided)</p> <ul style="list-style-type: none"> • Movement ABC test battery for motor performance (coordination, fine motor skills) • Developmental Coordination Disorder Questionnaire for behavior • Wechsler Preschool and Primary Scale of Intelligence, Revised for intelligence (total, verbal, performance) • Neuropsychological Assessment (NEPSY-II) for visual perception, visuomotor integration, inhibitory control • Rey’s Auditory Verbal Learning test (verbal memory) • Test of Everyday Attention for Children (attention) <p>Behavioral tests (references for procedure provided)</p> <ul style="list-style-type: none"> • Child Behavior Checklist and Teacher’s Report Form • Attention Deficit/Hyperactivity Disorder questionnaire <p>Analysis: Pearson correlation (for normally distributed variables) or Spearman’s rank correlation (for non-normally distributed variables)</p> <p>Data quality: Medium (1.8)</p>	<p>(Correlations of similar, but somewhat smaller, magnitude were seen between PCB-153 or 4,4-DDE and coordination; none of the other nine compounds examined were associated with either intelligence measure.)</p> <p>Results for correlations between HBCD and other neuropsychological and behavioral outcomes were not shown, but were stated to be not statistically significant ($p > 0.10$).</p>														
<p><i>Studies in adolescents, neurodevelopment</i></p>															
<p>Kiciński et al. (2012) (Belgium, 2008–2011)</p> <p>Population: 515 adolescents (13–17 yrs old) residing in two industrial areas and randomly selected from the general population; participation rates 22–34% in the three groups; sample size varied by test (designed as “biomonitoring program for environmental health surveillance”)</p> <p>Exposure measures: Serum samples, HBCD >75% were less than the LOQ (LOQ = 30 ng/L); Median <30 ng/L (range: <LOQ–234) ng/L</p> <p>Effect measures: Neurobehavior (Neurobehavioral Evaluation System, NES-3), computerized battery (references for procedure provided)</p> <p>Continuous Performance test (attention)</p> <p>Digit-Symbol test (visual scanning and information processing)</p> <p>Digit Span test (working memory)</p> <p>Finger Tapping (motor function)</p> <p>Analysis: Regression models (linear or negative binomial depending on outcome)</p> <p>Data quality:^a Medium (1.9)</p>	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 70%;"></th> <th style="text-align: right;">Beta (95% CI)^b</th> </tr> </thead> <tbody> <tr> <td>Continuous Performance reaction time (msec) (n = 489)</td> <td style="text-align: right;">–3.53 (–18.72, 11.67)</td> </tr> <tr> <td>Continuous Performance errors of omission (%) (n = 489)</td> <td style="text-align: right;">27.8 (–17.5, 97.9)</td> </tr> <tr> <td>Continuous Performance errors of commission (%) (n = 489)</td> <td style="text-align: right;">21.8 (–2.5, 52.2)</td> </tr> <tr> <td>Digit Symbol total latency (sec) (n = 340)</td> <td style="text-align: right;">–0.44 (–6.59, 5.72)</td> </tr> <tr> <td>Digit Span, Forward (n = 511)</td> <td style="text-align: right;">0.13 (–0.22, 0.49)</td> </tr> <tr> <td>Digit Span, Backward (n = 499)</td> <td style="text-align: right;">–0.04 (–0.39, 0.31)</td> </tr> </tbody> </table> <p>Linear regression models for all outcomes except Continuous Performance errors of omission and commission, where negative binomial models were used. All models adjusted for age, gender, type of education, blood lipids, smoking, parental smoking, parental education, and parental home ownership. Additional covariates evaluated included BMI, physical activity, computer use, alcohol and fish consumption, blood lead, and blood PCBs, and were included based on a stepwise regression procedure.</p>		Beta (95% CI) ^b	Continuous Performance reaction time (msec) (n = 489)	–3.53 (–18.72, 11.67)	Continuous Performance errors of omission (%) (n = 489)	27.8 (–17.5, 97.9)	Continuous Performance errors of commission (%) (n = 489)	21.8 (–2.5, 52.2)	Digit Symbol total latency (sec) (n = 340)	–0.44 (–6.59, 5.72)	Digit Span, Forward (n = 511)	0.13 (–0.22, 0.49)	Digit Span, Backward (n = 499)	–0.04 (–0.39, 0.31)
	Beta (95% CI) ^b														
Continuous Performance reaction time (msec) (n = 489)	–3.53 (–18.72, 11.67)														
Continuous Performance errors of omission (%) (n = 489)	27.8 (–17.5, 97.9)														
Continuous Performance errors of commission (%) (n = 489)	21.8 (–2.5, 52.2)														
Digit Symbol total latency (sec) (n = 340)	–0.44 (–6.59, 5.72)														
Digit Span, Forward (n = 511)	0.13 (–0.22, 0.49)														
Digit Span, Backward (n = 499)	–0.04 (–0.39, 0.31)														

	Effects of levels above the LOQ were estimated. Models evaluating number of digits in Digital Span test were also adjusted for the method of test administration.
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^aBased on OPPT data evaluation criteria

^bBeta is for HBCD >30 ng/L (LOQ) versus <30 ng/L; 0.0 = no association.

Table 1-9. Evidence pertaining to neurological effects in animals following developmental exposure to HBCD

Reference and study design	Results				
<i>Neurodevelopmental milestones</i>					
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Data quality: ^d High (0)	Doses (mg/kg-d)				
	F1 offspring^a	0	17	168	1,570
	F2 offspring^a	0	15	139	1,360
	Surface righting reflex response time (s)				
	Male, F1, PND 5 (n = 17–24 litters)				
	Mean (SD)	2.3 (1.1)	2 (0.6)	1.8 (0.5)	1.6* (0.3)
	% of control ^b	–	–13%	–22%	–30%
	Female, F1, PND 5 (n = 17–23 litters)				
	Mean (SD)	3.1 (1.8)	2.4 (1.5)	2.9 (2.6)	2.6 (2.6)
	% of control ^b	–	–23%	–6%	–16%
	Male, F2, PND 5 (n = 19–22 litters)				
	Mean (SD)	2.1 (1.7)	2.0 (1.5)	2.8 (2.5)	2.2 (2.3)
	% of control ^b	–	–5%	33%	5%
	Female, F2, PND 5 (n = 16–22 litters)				
Mean (SD)	2.3 (0.9)	2.4 (1.7)	2.1 (0.9)	3.7 (3.7)	
% of control ^b	–	4%	–9%	61%	
Mid-air righting reflex completion rate (%)					
Male, F1, PND 18 (n = 17–23 litters)					
Mean	100	100	100	100	
% of control ^b	–	0%	0%	0%	
Female, F1, PND 18 (n = 17–23 litters)					
Mean	100	100	100	100	
% of control ^b	–	0%	0%	0%	
Male, F2, PND 18 (n = 13–22 litters)					
Mean	100	100	94.4	100	
% of control ^b	–	0%	–6%	0%	
Female, F2, PND 18 (n = 13–21 litters)					
Mean	100	100	90	76.9*	
% of control ^b	–	0%	–10%	–23%	
Miller-Rhodes et al. (2014) Rats, Long-Evans	Doses (mg/kg-d)				
		0	3	10	30
Age at which 85% of pups could perform righting reflex					

Reference and study design	Results				
Gavage F1: Continuous maternal exposure throughout gestation Data quality: ^d Medium (2)*	Male, F1 (n = 8–10 litters)				
	PND	5	5	5	3
	% of control ^b	–	0%	0%	–40%
	Female, F1 (n = 8–10 litters)				
	PND	7	5	5	3
	% of control ^b	–	–29%	–29%	–57%
	FOB including the righting reflex was conducted every other day from PND 1 to 21. Every pup in each litter was examined.				
	Animals that did not respond to tail pinch (mean % pups per litter)				
	Males and females, F1 PNDs 1–21 (n = 8–10 litters)				
	Mean (SE)	39 (2)	28* (2)	31* (2)	27* (2)
% of control ^b	–	–28%	–21%	–31%	
Grip strength (Newtons)					
Male, F1, PND 26 (n = 8–10 litters)					
Mean (SE)	4.1 (0.2)	3.9 (0.2)	2.8* (0.2)	3.3* (0.2)	
% of control ^b	–	–5%	–32%	–20%	
Data for tail pinch and grip strength were digitized from figure. No significant treatment-related effect on grip strength in females.					
<i>Executive function and locomotor activity</i>					
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation	Doses (mg/kg-d)				
	Male, F1	0	11	115	1,142
	Female, F1	0	14	138	1,363
	Locomotor activity				
F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Data quality: ^d High (1.0)	Male, F1, PNW 4 (n = 10)				
		Mean (SD) % of control ^b			
	0–10 min	141.9 (63.5)	240.9 (116.7)	127.4 (79.2)	162.4 (124.9)
		–	70%	–10%	14%
	10–20 min	86.1 (59.3)	116.8 (86.3)	71.7 (44.4)	53.3 (53.7)
		–	36%	–17%	–38%
	20–30 min	39.9 (49.4)	58.2 (66.8)	11.8 (11.4)	8.8 (13.9)
		–	46%	–70%	–78%
	30–40 min	15.6 (19.1)	29.5 (45.0)	2.9 (5.9)	7.1 (11.9)
		–	89%	–81%	–54%
	40–50 min	13.8 (21.5)	5.7 (18.0)	0.0 (0.0)	1.0 (2.5)
		–	–59%	–100%	–93%
	50–60 min	4.8 (15.2)	0.8 (2.5)	0.0 (0.0)	5.7 (18.0)
	–	–83%	–100%	19%	
	Female, F1, PNW 4 (n = 10)				
	Mean (SD) % of control ^b				
0–10 min	196.9 (75.8)	194.1 (112.7)	176.7 (93.8)	172.6 (101.9)	

Reference and study design	Results				
		–	–1%	–10%	–12%
	10–20 min	77.6 (50.0)	70.7 (64.3)	84.7 (66.2)	35.2 (31.8)
		–	–9%	9%	–55%
	20–30 min	40.4 (44.7)	52.1 (62.3)	39.5 (49.4)	17.7 (31.2)
		–	29%	–2%	–56%
	30–40 min	13.0 (30.9)	15.4 (42.0)	5.6 (12.3)	15.8 (22.0)
		–	18%	–57%	22%
	40–50 min	5.4 (14.2)	2.3 (7.3)	9.9 (31.3)	3.6 (11.4)
		–	–57%	83%	–33%
	50–60 min	0.8 (1.9)	1.3 (3.5)	4.9 (12.4)	5.0 (11.2)
		–	63%	513%	525%
	T-maze swim test, trial time (s)				
	Male, F1, PNW 6 (n = 10)				
		Mean (SD) % of control ^b			
	Day 1	8.3 (2.5)	8.0 (1.1)	6.9 (1.3)	8.3 (2.5)
		–	–4%	–17%	0%
	Day 2	48.7 (19.1)	43.5 (18.4)	33.2 (12.0)	40.8 (17.4)
		–	–11%	–32%	–16%
	Day 3	38.9 (14.8)	27.8 (8.8)	32.4* (37.3)	18.4* (4.9)
		–	–29%	–17%	–53%
	Day 4	27.5 (12.3)	30.4 (12.3)	28.0 (24.7)	19.6 (5.2)
		–	11%	2%	–29%
	Female, F1, PNW 6 (n = 10)				
		Mean (SD) % of control ^b			
	Day 1	12.2 (4.7)	10.8 (4.0)	8.8 (4.4)	10.5 (2.3)
		–	–11%	–28%	–14%
	Day 2	49.1 (18.2)	43.4 (17.1)	40.7 (14.2)	39.2 (12.2)
		–	–12%	–17%	–20%
	Day 3	42.1 (32.6)	35.1 (15.8)	34.5 (23.3)	31.5 (19.4)
		–	–17%	–18%	–25%
	Day 4	28.3 (8.1)	31.6 (19.6)	30.7 (13.0)	25.4 (10.1)
		–	12%	8%	–10%
Miller-Rhodes et al. (2014)	Doses (mg/kg-d)				
		0	3	10	30
Rats, Long-Evans Gavage	Go/no-go task (% hits)				
F1: Continuous maternal	Males and females, F1 (n = 4)				
	Mean (SE)	94.8 (0.7)	87.8 (1.9)*	94.1 (1.6)	94.8 (0.9)
	% of control ^b	–	–7%	–1%	0%

Reference and study design	Results				
exposure throughout gestation Go/no-go task: animals tested on PNM 14 and 21 RR task animals tested on PNM 11 and 19 Data quality: ^d Medium (2)*	Random ratio (RR) task (responses per minute)				
	Males and females, F1 (n = 4)				
			Mean (SD) % of control ^b		
	RR1	8.6 (1.5)	7.5 (0.1)	7.6 (1.2)	8.5 (1.2)
		–	–13%	–12%	–1%
	RR2	14.1 (2.6)	12.8 (1.8)	12.5 (1.5)	14.9 (1.7)
		–	–9%	–11%	6%
	RR5	20.1 (4.0)	20.2 (2.8)	18.9 (2.9)	22.7 (1.5)
		–	1%	–6%	13%
	RR10	26.9 (3.7)	26.4 (4.0)	23.0 (3.6)	25.9 (3.2)
	–	–2%	–15%	–4%	
RR20	24.7 (4.5)	26.5 (3.7)	23.6 (5.3)	30.6 (2.9)	
	–	7%	–4%	24%	
All data were digitized from figure. Go/no-go task: hit defined as lever press behavior during a “go” trial. RR task: Different schedules (e.g., RR1, RR2...) correspond to the average number of lever presses between reinforcements.					
Eriksson et al. (2006) Mice, NMRI Gavage F1: single dose on PND 10 Males only Data quality: ^d Medium (2)*	Doses (mg/kg)				
		0	0.9	13.5	
	Horizontal locomotion (beam hits)				
	Male, F1, PNM 3 (n = 10)				
			Mean (SD) % of control ^b		
	0–20 min	499 (81)	414* (50)	213* (58)	
		–	–17%	–57%	
	20–40 min	209 (62)	256 (50)	232 (39)	
		–	22%	11%	
	40–60 min	12 (8)	12 (16)	256* (47)	
	–	0%	2,103%		
Rearing (beam hits)					
Male, F1, PNM 3 (n = 10)					
		Mean (SD) % of control ^b			
0–20 min	1,596 (285)	1,206* (260)	322*(78)		
	–	–24%	–80%		
20–40 min	487 (91)	525 (143)	485 (130)		
	–	8%	0%		
40–60 min	104 (13)	142 (13)	480* (104)		
	–	37%	362%		
Total activity (beam hits)					
Male, F1, PNM 3 (n = 10)					
Mean (SD)					

Reference and study design	Results			
	% of control ^b			
	0–20 min	4,741 (606)	4,491 (535)	2,495* (321)
		–	–5%	–47%
	20–40 min	2,210 (428)	2,424 (606)	2,566 (321)
		–	10%	16%
	40–60 min	1,176 (214)	998 (214)	2,709* (570)
		–	–15%	130%
	Morris water maze (s)			
	Male, F1, PNM 3 (n = 12–17)^c			
		Mean % of control ^b		
Day 1	27	27	25	
	–	0%	–1%	
Day 2	20	21	23	
	–	8%	18%	
Day 3	15	17	19	
	–	13%	24%	
Day 4	10	14*	20*	
	–	33%	90%	
Day 5	14	20	21*	
	–	46%	54%	
All data were digitized from figure. Morris water maze: error data not shown. Day 5, platform relocated.				
<i>Other neurological effects</i>				
Ema et al. (2008)	Doses (mg/kg-d)			
Rats, CRL:CD(SD)	F1 offspring^a	0	17	168
Diet	Male, F1	0	11	115
Two generation	Female, F1	0	14	138
F0: exposure started 10 wks prior to mating	F2 offspring^a	0	15	139
F1: dietary exposure post weaning until necropsy	Absolute brain weight (mg)			
F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	Male, F1 PND 26 (n = 17–23)			
	Mean (SD)	1.64 (0.09)	1.66 (0.05)	1.62 (0.07)
	% of control ^b	–	1%	–1%
				–5%
	Female, F1 PND 26 (n = 14–23)			
	Mean (SD)	1.58 (0.09)	1.61 (0.07)	1.59 (0.08)
	% of control ^b	–	2%	1%
				–4%
	Male, F1 adult (n = 22–24)			
	Mean (SD)	2.18 (0.08)	2.22 (0.08)	2.18 (0.09)
	% of control ^b	–	2%	0%
				–3%
	Female, F1 adult (n = 13–22)			
Data quality:^d High (1.0)				

Reference and study design	Results									
	Mean (SD)	2.07 (0.09)	2.06 (0.07)	2.06 (0.08)	1.97* (0.06)					
	% of control ^b	–	0%	0%	–5%					
	Male, F2 PND 26 (n = 13–22)									
	Mean (SD)	1.62 (0.13)	1.65 (0.08)	1.60 (0.10)	1.46* (0.09)					
	% of control ^b	–	2%	–1%	–10%					
	Female, F2 PND 26 (n = 13–22)									
Mean (SD)	1.57 (0.11)	1.58 (0.07)	1.55 (0.12)	1.41* (0.15)						
% of control ^b	–	1%	–1%	–10%						
Lilienthal et al. (2009)	Doses (mg/kg-d)									
Rats, Wistar Diet	0	0.1	0.3	1	3	10	30	100		
	BAEPs, click threshold (dB)									
F0: exposure started 10 wks (male) or 2 wks (female) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning until sacrifice (~PNW 20)	Male, F1, PNW 20 (n = 4–6)**									
	Mean (SE)	47 (2)	47 (4)	40 (2)	49 (7)	48 (8)	48 (4)	53 (3)	56 (4)	
	% of control ^b	–	0%	–15%	4%	2%	2%	13%	19%	
	Female, F1, PNW 20 (n = 4–6)									
	Mean (SE)	44 (3)	47 (2)	53 (4)	52 (3)	41 (3)	54 (2)	49 (2)	48 (2)	
	% of control ^b	–	7%	20%	18%	–7%	23%	11%	9%	
Data quality: ^d High (1.3)	Data for males were digitized from figure.									
	Catalepsy, box, foreleg latency (s)									
	Male, F1, PNW 15 (n = 5)**									
Mean (SE)	135 (24)	150 (18)	105 (19)	98 (26)	129 (27)	140 (27)	99 (33)	69 (30)		
% of control ^b	–	11%	–22%	–27%	–4%	4%	–27%	–49%		
	Female, F1, PNW 15 (n = 5)**									
Mean (SE)	136 (24)	77 (28)	128 (32)	145 (34)	111 (31)	65 (38)	56 (25)	60 (30)		
% of control ^b	–	–43%	–6%	7%	–18%	–52%	–59%	–56%		
	Data for females were digitized from figure.									
van der Ven et al. (2009)	Doses (mg/kg-d)									
Rats, Wistar Diet One generation	0	0.1	0.3	1	3	10	30	100		
	Absolute brain weight (g)									
F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous	Male, F1, PNW 11 (n = 4–5)**									
	Mean (SE)	1.84 (0.12)	1.87 (0.07)	1.94 (0.06)	1.98 (0.07)	1.91 (0.07)	1.88 (0.05)	1.92 (0.06)	1.78 (0.06)	
	% of control ^b	–	2%	5%	8%	4%	2%	4%	–3%	

Reference and study design	Results									
cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Data quality:^d High (1.2)	Female, F1, PNW 11 (n = 4-5)									
	Mean (SE)	1.76 (0.14)	1.71 (0.09)	1.71 (0.09)	1.77 (0.08)	1.62 (0.23)	1.80 (0.06)	1.76 (0.08)	1.66 (0.07)	
	% of control ^b	-	-3%	-3%	1%	-8%	2%	0%	-6%	

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.

**Significant dose response trend as reported by study authors.

^aF1 and F2 offspring doses presented as mean maternal gestational F0 and F1 doses, respectively.

^bPercent change compared to control calculated as: (treated value - control value)/control value × 100.

^cExact number of animals examined per dose group was unclear based on the published paper.

^dBased on OPPT data evaluation criteria. *[Miller-Rhodes et al. \(2014\)](#) was downgraded to a Medium. The calculated score was 1.4. [Eriksson et al. \(2006\)](#) was also downgraded to a Medium. The calculated score was 1.3

PNM = postnatal month

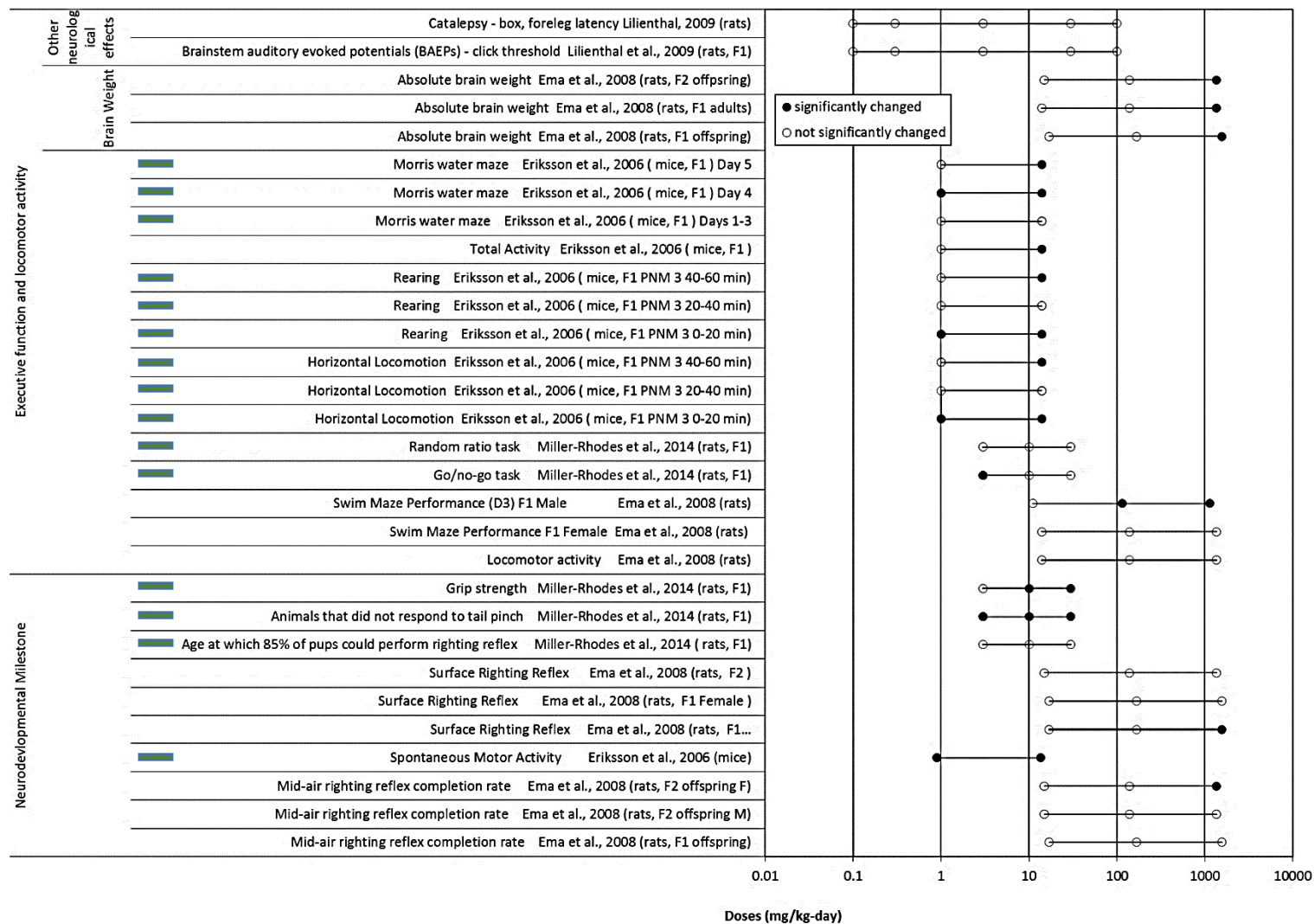


Figure 1-7. Exposure response array of nervous system effects following oral exposure. [Lilienthal et al. \(2009\)](#) and [Ema et al. \(2008\)](#) scored a High in data quality evaluation. [Miller-Rhodes et al. \(2014\)](#) and [Eriksson et al. \(2006\)](#) scored a Medium (indicated with).

1.5.4 Mechanistic Evidence

1.5.4.1 Thyroid Perturbation and Neurodifferentiation

Thyroid hormones are known to play a key role in development of the vertebrate central nervous system, and perinatal exposure to thyroid-disrupting chemicals has been shown to have lasting effects on cognitive and behavioral outcomes ([Gilbert et al., 2012](#); [Howdeshell, 2002](#); [Koibuchi and Chin, 2000](#)). The evidence to support mechanisms by which HBCD may affect thyroid hormones is covered elsewhere (Section 1.2.1, Mechanistic Evidence); therefore, the following discussion focuses on the available studies that specifically investigated possible associations between HBCD-mediated thyroid hormone perturbation and neurodevelopmental endpoints ([Fujimoto et al., 2013](#); [Saegusa et al., 2012](#); [Ibhazehiebo et al., 2011a](#); [Ibhazehiebo et al., 2011b](#)).

As discussed in Section 1.2.1, HBCD elicited a decrease in thyroid hormone levels in developmentally exposed rats ([Saegusa et al., 2009](#)). In two follow-up studies by the same research group, thyroid perturbation corresponded with several changes in brain morphometry indicative of altered neuronal migration and neurogenesis in the hippocampus, a region that is critical for learning and memory ([Fujimoto et al., 2013](#); [Saegusa et al., 2012](#)). Developmental exposure also elicited a statistically significant increase in the number of astrocytes and oligodendrocytes in the cingulum, an area of the brain involved in regulating behaviors related to emotion and cognitive function ([Fujimoto et al., 2013](#)). These results mirror those previously found following developmental exposure to known anti-thyroid drugs, propylthiouracil and methimazole ([Fujimoto et al., 2012](#)). These data are supported by two studies with primary rat neuronal cell cultures. During normal development, thyroid hormones regulate neurite growth and arborization of cerebellar granule neurons (CGNs) and Purkinje cells. In the cerebellum, these cells generate a highly interconnected dendritic network that is critical for motor control and coordination ([Gilbert et al., 2012](#); [Koibuchi and Chin, 2000](#)). Primary rat Purkinje cell ([Ibhazehiebo et al., 2011a](#)) and CGN ([Ibhazehiebo et al., 2011b](#)) cultures co-exposed to thyroid hormone and sub-nanomolar concentrations of α -HBCD showed statistically significant reductions in thyroid hormone-induced neurite growth and arborization. These effects were seen at concentrations several orders of magnitude below those that reduced viability by >50% in rat primary CGNs ([Reistad et al., 2006](#)) and human neuroblastoma cells ([Al-Mousa and Michelangeli, 2012](#)), indicating that they were not due to cytotoxicity. HBCD-mediated effects on neurite growth and arborization could be ameliorated by elevated thyroid hormone levels ([Ibhazehiebo et al., 2011a](#)) or coexposure with brain-derived neurotrophic factor ([Ibhazehiebo et al., 2011b](#)).

1.5.4.2 Calcium Homeostasis

Several studies suggest that HBCD may alter calcium (Ca²⁺) homeostasis in the brain by affecting three types of calcium transporters: sarco-endoplasmic reticulum Ca²⁺-dependent ATPase (SERCA) pumps ([Al-Mousa and Michelangeli, 2014, 2012](#)), ligand-gated Ca²⁺ channels (LGCC) ([Reistad et al., 2006](#)), and voltage-gated Ca²⁺ channels (VGCC) ([Dingemans et al., 2009](#)). Within neurons, Ca²⁺ levels are typically maintained at low concentrations relative to the extracellular fluid; however, rapid influx can occur through various ion channels. After an influx event, low cytosolic Ca²⁺ levels are restored via active transport across the cell membrane or sequestration into subcellular compartments. Tight regulation of Ca²⁺ is critical as both excess and insufficient levels can adversely affect numerous cellular processes.

SERCA uses ATP to actively transport excess Ca^{2+} from the cytosol into intracellular compartments to regulate protein synthesis and neurotransmitter release ([Neher and Sakaba, 2008](#); [Rodriguez et al., 2001](#)). HBCD increased intracellular Ca^{2+} and cell death in human neuroblastoma cells (SH-SY5Y) via concentration-dependent SERCA inhibition ([Al-Mousa and Michelangeli, 2014, 2012](#)). HBCD interacts with SERCA in a manner that: (1) reduces ATP binding affinity and (2) stabilizes the low Ca^{2+} affinity conformation ([Al-Mousa and Michelangeli, 2014](#)). Exposure of PC12 cells to either the technical mixture or individual HBCD isomers reduced Ca^{2+} influx through VGCCs, but did not affect resting intracellular Ca^{2+} levels ([Dingemans et al., 2009](#)). γ -HBCD showed the greatest potency, whereas the α -isomer had a moderate effect similar to that of the technical mixture. These effects were associated with decreased catecholamine release, likely due to low cytosolic Ca^{2+} levels that were insufficient to trigger synaptic release ([Neher and Sakaba, 2008](#)). HBCD may also act as a mild LGCC-agonist. Co-exposure to MK801, an LGCC antagonist, was found to ameliorate HBCD-induced cytotoxicity, suggesting a role of this Ca^{2+} channel in neurotoxicity. Although no significant changes in intracellular Ca^{2+} calcium were reported, this was the only study that measured Ca^{2+} effects as an average across all cells, which may have reduced the sensitivity when compared to single cell measurements ([Al-Mousa and Michelangeli, 2012](#); [Dingemans et al., 2009](#)).

1.5.4.3 Neurotransmitter Reuptake

Adult male mice exposed to 25 mg/kg-day for 30 days showed decreased striatal levels of dopamine transporter and vesicular monoamine transporter 2, regulators of dopamine homeostasis and neurotransmission ([Genskow et al., 2015](#)). Similarly, an in vitro study found a dose-related reduction in dopamine and gamma-aminobutyric acid uptake in rat synaptosomes and vesicles exposed to HBCD ([Mariussen and Fonnun, 2003](#)). Although prolonged deficits in reuptake mechanisms could result in excessive stimulation of the post synaptic cell or deplete neurotransmitter stores in the presynaptic cell, [Genskow et al. \(2015\)](#) did not find significant changes in tissue concentrations of dopamine or its metabolites in adult mice exposed for 30 days.

1.6 Immune System Effects

1.6.1 Human Evidence

The potential for HBCD to affect the immune system has not been investigated in humans.

1.6.2 Animal Evidence

The potential for HBCD to affect the immune system has been examined in eight studies in rats ([Hachisuka et al., 2010](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#); [van der Ven et al., 2006](#); [WIL Research, 2001, 1997](#)) and mice ([Maranghi et al., 2013](#); [Watanabe et al., 2010](#)), with exposures ranging from a 28-day exposure in adults to continuous exposure across two generations.

Discussion of immune-related effects of HBCD is organized first by age of exposure (i.e., developmental or adult) and second by the type of endpoint evaluated (i.e., functional or observational). Exposure timing is an important factor that may influence the effect of chemical exposure on immune function, particularly for early-life exposure studies. In rodents, immune development occurs in a series of discrete stages until approximately PND 42. The developing

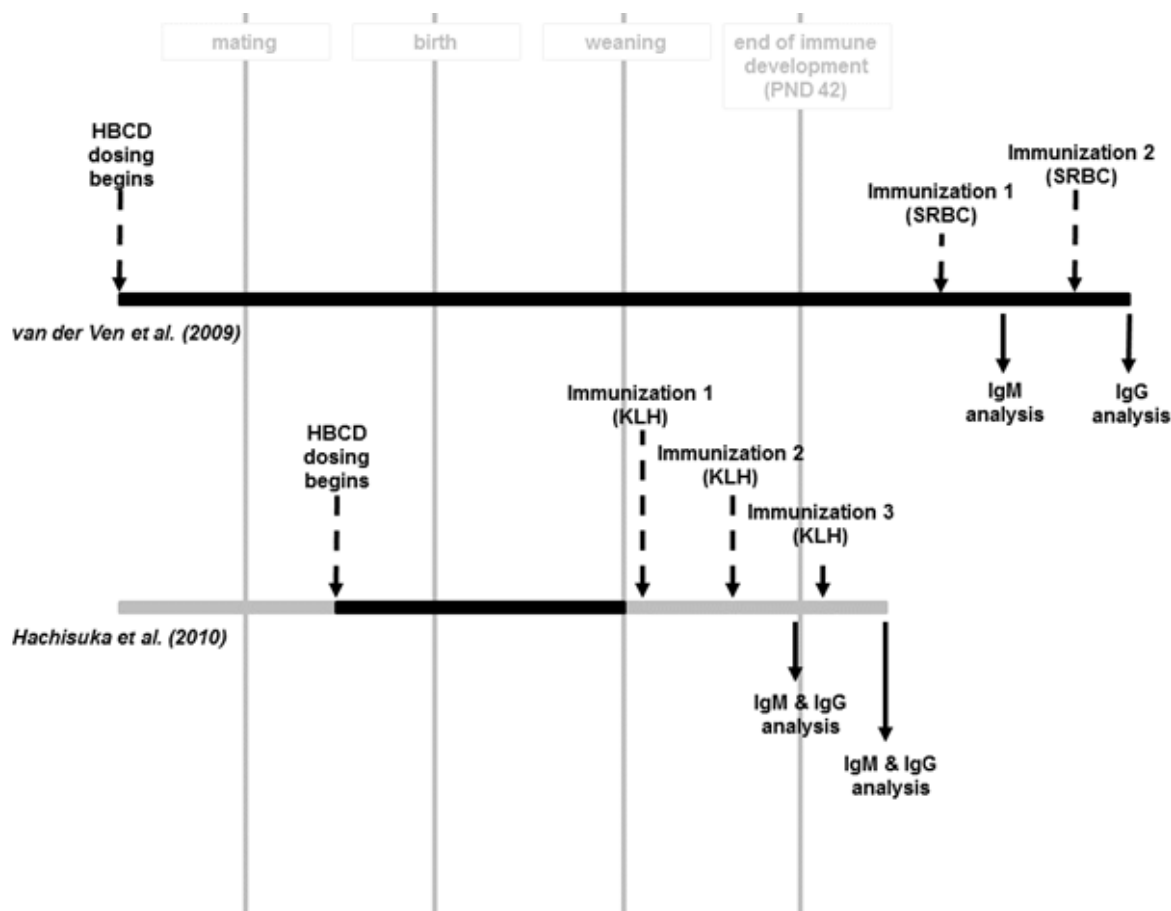
immune system is susceptible to perturbation resulting from chemical exposure, and exposures during this period may result in distinct toxicological consequences that would not be observed in animals exposed only as adults ([Burns-Naas et al., 2008](#)). With regard to the type of endpoint evaluated, functional immune outcomes, including response to challenge with an infectious agent or immunization with a foreign antigen, are the most relevant and sensitive for determining potential immunotoxicity because the primary role of the immune system is to protect host integrity from foreign challenge and potential insult. Laboratory animals are housed in environments that limit their exposure to antigenic stimulation or infectious agents, and their immune systems are typically in a resting state ([Who, 2012](#)). In the absence of a foreign challenge, observational endpoints, including structural alterations or changes in immune cell populations, can provide information about immune system effects, but are considered less sensitive and predictive ([Luster et al., 2005](#)).

A summary of the evidence pertaining to functional and observational immune system effects in experimental animals is presented in Table 1-10, Table 1-11, Table 1-12 and Figure 1-9. Studies are ordered within effect categories by decreasing exposure duration and then species.

1.6.2.1 Developmental Exposure

Functional immune Effects

Changes in functional immune endpoints (immunoglobulin G [IgG] and immunoglobulin [IgM] antibody production in response to foreign antigens) following developmental HBCD exposures were evaluated in two one-generation reproductive toxicity studies in male ([van der Ven et al., 2009](#)) or female rats ([Hachisuka et al., 2010](#)) (see Table 1-10 and Figure 1-8). Statistically significant changes in IgG levels were reported in both studies, but with opposite directions of effect; males exposed to up to 100 mg/kg-day showed a dose-dependent increase in IgG, whereas females exposed to approximately 1,500 mg/kg-day showed a decrease. Differences in the design of these two studies, including timing of exposure, immune challenge, and titer measurement (Figure 1-3), may have contributed to the inconsistent results. IgM activity was unaffected in [van der Ven et al. \(2009\)](#) and results were not reported by [Hachisuka et al. \(2010\)](#). [van der Ven et al. \(2009\)](#) also evaluated natural killer (NK) cell activity and found no treatment-related effects.



KLH = keyhole limpet hemocyanin; SRBC = sheep red blood cell

Horizontal lines represent the experimental timelines, with black indicating the time period when HBCD was administered (i.e., from 2 weeks prior to mating through IgG analysis in [van der Ven et al. \(2009\)](#), and from GD 10 to PND 21 in [Hachisuka et al. \(2010\)](#)).

Figure 1-8. Comparison of study designs used by [van der Ven et al. \(2009\)](#) and [Hachisuka et al. \(2010\)](#).

Observational Immune Effects

Five studies evaluated effects on observational immune parameters, including organ weights, hematology, and histopathology, in developmentally-exposed rats ([Hachisuka et al., 2010](#); [Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#)) or mice ([Maranghi et al., 2013](#)) (see Table 1-4 and Figure 1-4).

Thymus weights showed significant dose-response trends in male and female adult rats (PNW 11) continuously exposed to HBCD at doses up to 100 mg/kg-day ([van der Ven et al., 2009](#)) and in female F2 weanlings exposed to approximately 1,300 mg/kg-day HBCD throughout gestation and lactation ([Ema et al., 2008](#)). Spleen weight was reduced in both male and female F2 weanlings from the 1,300 mg/kg-day dose group ([Ema et al., 2008](#)). A significant positive trend was also reported for absolute popliteal lymph node weight in PNW 11 male, but not female, rats ([van der Ven et al., 2009](#)). No other treatment-related effects were reported for thymus

([Maranghi et al., 2013](#); [Hachisuka et al., 2010](#); [Saegusa et al., 2009](#)) or spleen weights ([Maranghi et al., 2013](#); [Hachisuka et al., 2010](#); [Saegusa et al., 2009](#); [van der Ven et al., 2009](#)).

Hematological analyses revealed significant treatment-related effects on several blood immune cell populations, although the pattern of effect was variable across studies, sex, and time point. Total white blood cell (WBC) count was measured in three studies. [Hachisuka et al. \(2010\)](#) reported statistically significant increases in WBC count in HBCD-exposed male rats on PNWs 3 and 11 (approximately 8 weeks after the end of the exposure). In contrast, [van der Ven et al. \(2009\)](#) reported a significant dose-related decrease in continuously exposed PNW 11 male rats, and [Ema et al. \(2008\)](#) found no effect on total WBCs of F1 males or females. In addition to total WBCs, several subpopulations were measured. [van der Ven et al. \(2009\)](#) found a significant dose-related increase and decrease in the fraction of neutrophils and lymphocytes, respectively. The magnitude of the lymphocyte change was small ($\leq 4\%$ change from control) and the biological significance is unclear. [Hachisuka et al. \(2010\)](#) also measured subpopulations of several leukocyte subtypes. On PNW 3, high-dose (1,505 mg/kg-day HBCD) male rats showed a decrease in activated T-cell and NK cell fractions and an increase in inactive B-cell fractions; however, cell fractions returned to control levels by PNW 11.

[Hachisuka et al. \(2010\)](#) and [van der Ven et al. \(2009\)](#) reported inconsistent effects on splenic NK and cytotoxic T-cell populations. [Hachisuka et al. \(2010\)](#) reported a statistically significant decrease in the NK cell fraction (e.g., CD4NKT cells, PNW 3) and an increase in the cytotoxic T-cell fraction in adult rats (CD8+ cells, PNW 11) that were gestationally and lactationally exposed to HBCD. In contrast, male rats continuously exposed through PNW 11 showed a dose-dependent increase in the NK cell fraction and no change in the cytotoxic T-cell fraction. No other treatment-related effects were observed for other immune cell counts in the spleen ([van der Ven et al., 2009](#)).

Immune cell counts were also measured in the thymus ([Hachisuka et al., 2010](#)) and bone marrow ([van der Ven et al., 2009](#)). Rats showed decreases in the thymus fraction of active and regulatory T-cells and an increase in NK cells on PNW 3 and PNW 11, respectively ([Hachisuka et al., 2010](#)). WBC counts in bone marrow showed an increasing dose-related trend in adult males continuously exposed to HBCD at doses up to 100 mg/kg-day ([van der Ven et al., 2009](#)).

Histological examination of immune-related tissues showed limited changes with no clear pattern of effect. Thymus tissues showed increased incidence of “starry sky” appearance ([Hachisuka et al., 2010](#)) and blurring of the corticomedullary demarcation ([Maranghi et al., 2013](#)) in rats and mice, respectively. In the spleen, increased incidence of marginal zone enlargement was also observed in adult (PNW 11) rats continuously exposed to 100 mg/kg-day HBCD ([van der Ven et al., 2009](#)). No other treatment-related histological changes were observed ([Hachisuka et al., 2010](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#)).

1.6.2.2 Adult Exposure

Functional Immune Effects

Two studies evaluated functional immune endpoints following adult exposure to HBCD for 28 days ([Watanabe et al., 2010](#); [van der Ven et al., 2006](#)). No statistically significant changes were

observed in NK cell activity in adult male rats ([van der Ven et al., 2006](#)) or host immunity infection in female mice ([Watanabe et al., 2010](#)).

Observational Immune Effects

Treatment related effects on organ weight, hematology, and histopathology were evaluated in four rat studies ([Ema et al., 2008](#); [van der Ven et al., 2006](#); [WIL Research, 2001, 1997](#)) (see Table 1-5 and Figure 1-4). Trends identified by the authors as statistically significant were reported for absolute thymus weight in male rats and for absolute spleen weight in female rats administered up to 200 mg/kg-day for 28 days ([van der Ven et al., 2006](#)). In both cases, effects were not consistent across sexes, the magnitude of the effect was small, and the biological significance of these changes is unclear. Hematological analyses revealed a statistically significant reduction in the percentage of stabform and segmented neutrophils and increase in the lymphocyte fraction of F0 females exposed to HBCD for 14 weeks ([Ema et al., 2008](#)); however, these effects were only seen in the low-dose group (approximately 14 mg/kg-day) in this study and not in a second study involving adult exposure ([van der Ven et al., 2006](#)). Total splenocyte number was decreased in adult male rats in the 28-day study by [van der Ven et al. \(2006\)](#). No other observational immune endpoints were affected ([Ema et al., 2008](#); [WIL Research, 2001, 1997](#)).

Table 1-10. Evidence pertaining to functional immune system effects in animals following exposure to HBCD during development

Reference and study design	Results								
van der Ven et al. (2009) Rats, Wistar Diet One generation F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Data quality: ^c High (1.2)	Doses (mg/kg-d)								
	Male, F1	0	0.1	0.3	1	3	10	30	100
	SRBC antibody titers IgG (extinction)								
	Male, F1, PNW 11 (n = 2-4)**								
	Mean (SD)	0.182 (0.128)	0.362 (0.333)	0.174 (0.143)	0.233 (0.169)	0.152 (0.180)	0.444 (0.143)	0.856 (0.231)	0.469 (0.205)
% change ^a	-	99%	-4%	28%	-16%	144%	370%	158%	
Animals (males only) immunized with SRBCs on PNWs 8 and 10.									
Hachisuka et al. (2010) Rats, SD:IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11 Data quality: ^c Medium (1.9)	Doses (mg/kg-d)^b								
	Female, F1	0		14.8		146.3		1,505	
	Antibody IgG responses to KLH (titer)								
	Female, F1, PND 40 (n = 7-8, estimated from graph)								
	Mean	139,452		63,196		95,592		42,548*	
% change ^a	-		-55%		-31%		-69%		
Data were digitized from figure; animals (females only) challenged with KLH on PNDs 23 and 33. IgM titers (enzyme-linked immunosorbent assay) were measured on PND 40.									

*Statistically significantly different from the control at $p < 0.05$.

**Significant dose response trend.

^aPercent change compared to control calculated as: (treated value – control value)/control value × 100.

^bTWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PNDs 1–9, and PNDs 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = (8.1 mg/kg-day × 11 days) + (14.3 mg/kg-day × 10 days) + (21.3 mg/kg-day × 12 days)/33 days = 14.8 mg/kg-day.

^cBased on OPPT data evaluation criteria.

Table 1-11. Evidence pertaining to observational immune system effects in animals following exposure to HBCD during development

Reference and study design	Results				
<i>Organ weight</i>					
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	Doses (mg/kg-d)				
	F1 offspring^a	0	17	168	1,570
	Male, F1	0	11	115	1,142
	Female, F1	0	14	138	1,363
	F2 offspring^a	0	15	139	1,360
	Absolute spleen weight (mg)				
	Male, F1, adult (n = 22–24)				
	Mean (SD)	885 (168)	840 (147)	878 (163)	851 (113)
	% change ^b	–	–5%	–1%	–4%
	Male, F1, PND 26 (n = 17–23)				
	Mean (SD)	336 (62)	327 (41)	334 (43)	309 (69)
	% change ^b	–	–3%	–1%	–8%
	Female, F1, adult (n = 13–22)				
	Mean (SD)	632 (124)	595 (68)	624 (93)	578 (70)
	% change ^b	–	–6%	–1%	–9%
	Female, F1, PND 26 (n = 14–23)				
	Mean (SD)	311 (53)	306 (44)	304 (59)	280 (40)
	% change ^b	–	–2%	–2%	–10%
	Male, F2, PND 26 (n = 13–22)				
	Mean (SD)	360 (83)	361 (54)	346 (78)	263* (50)
	% change ^b	–	0%	–4%	–27%
	Female F2, PND 26 (n = 13–21)				
	Mean (SD)	325 (59)	302 (42)	299 (62)	225* (45)
	% change ^b	–	–7%	–8%	–31%
	Absolute thymus weight (mg)				
	Male, F1, adult (n = 22–24)				
	Mean (SD)	344 (72)	305 (92)	368 (100)	341 (76)
	% change ^b	–	–11%	7%	–1%
	Female, F1, adult (n = 13–22)				
	Mean (SD)	250 (62)	233 (62)	276 (80)	259 (76)
	% change ^b	–	–7%	10%	4%
	Male, F1, PND 26 (n = 17–23)				
	Mean (SD)	342 (68)	339 (50)	369 (59)	317 (57)
Data quality:^c High (1.0)					

Reference and study design	Results								
	% change ^b	–	–1%	8%	–7%				
	Female, F1, PND 26 (n = 14–23)								
	Mean (SD)	335 (64)	330 (58)	370 (58)	305 (31)				
	% change ^b	–	–1%	10%	–9%				
	Male, F2, PND 26 (n = 13–22)								
	Mean (SD)	343 (92)	336 (57)	360 (88)	282 (71)				
	% change ^b	–	–2%	5%	–18%				
	Female, F2, PND 26 (n = 13–22)								
	Mean (SD)	338 (85)	324 (50)	331 (69)	260* (80)				
	% change ^b	–	–4%	–2%	–23%				
van der Ven et al. (2009) Rats, Wistar Diet One generation F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Data quality: ^c High (1.2)	Doses (mg/kg-d)								
	0	0.1	0.3	1	3	10	30	100	
	Absolute popliteal lymph node weight (mg)								
	Male, F1 (n = 4–5)**								
	Mean (SD)	9 (2)	10 (3)	9 (4)	15 (11)	9 (3)	8 (1)	10 (5)	21 (16)
	% change ^b	–	11%	0%	67%	0%	–11%	11%	133%
	Female, F1 (n = 4–5)								
	Mean (SD)	8 (2)	9 (2)	9 (2)	8 (2)	8 (2)	8 (2)	9 (1)	7 (2)
	% change ^b	–	12%	12%	0%	0%	0%	12%	–12%
	Absolute spleen weight (g)								
	Male, F1 (n = 4–5)								
	Mean (SD)	0.49 (0.12)	0.53 (0.07)	0.49 (0.03)	0.58 (0.07)	0.49 (0.05)	0.50 (0.07)	0.58 (0.09)	0.48 (0.06)
	% change ^b	–	8%	0%	18%	0%	2%	18%	–2%
	Female, F1 (n = 4–5)								
	Mean (SD)	0.40 (0.04)	0.39 (0.04)	0.37 (0.06)	0.56 (0.37)	0.56 (0.42)	0.38 (0.05)	0.40 (0.04)	0.39 (0.07)
	% change ^b	–	–3%	–8%	40%	40%	–5%	0%	–3%
	Absolute thymus weight (g)								
	Male, F1 (n = 4–5)**								
	Mean (SD)	0.62 (0.10)	0.54 (0.12)	0.53 (0.12)	0.56 (0.13)	0.50 (0.09)	0.55 (0.08)	0.48 (0.14)	0.45 (0.06)
	% change ^b	–	–13%	–15%	–10%	–19%	–11%	–23%	–27%
	Female, F1 (n = 4–5)**								
	Mean (SD)	0.49 (0.07)	0.41 (0.05)	0.40 (0.04)	0.42 (0.05)	0.48 (0.10)	0.45 (0.06)	0.44 (0.11)	0.37 (0.07)
	% change ^b	–	–16%	–18%	–14%	–2%	–8%	–10%	–24%
Hachisuka et al. (2010) Rats, SD:IGS Diet F1: maternal exposure from	Doses (mg/kg-d)^c								
	0	15			146		1,505		
	Absolute spleen weight (g)								
	Male, F1, PNW 3 (n = 10)								
	Mean (SD)	0.29 (0.05)	0.25 (0.03)	0.22 (0.04)	0.23 (0.04)				
	% change ^b	–	–14%	–24%	–21%				

Reference and study design	Results								
GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11 Only males evaluated Data quality: ^e Medium (1.9)	Male, F1, PNW 11								
	Mean (SD)	0.55 (0.08)	0.55 (0.11)	0.56 (0.08)	0.53 (0.13)				
	% change ^b	–	0%	2%	–4%				
	Absolute thymus weight (g)								
	Male, F1, PNW 3 (n = 10)								
	Mean (SD)	0.21 (0.06)	0.24 (0.05)	0.21 (0.06)	0.21 (0.03)				
% change ^b	–	14%	0%	0%					
Male, F1, PNW 11 (n = 10)									
Mean (SD)	0.79 (0.08)	0.88 (0.17)	0.88 (0.18)	0.81 (0.13)					
% change ^b	–	11%	11%	3%					
<i>Hematology</i>									
Ema et al. (2008)	Doses (mg/kg-d)								
Rats, CRL:CD(SD)	Male, F1	0	11	115	1,142				
Diet	Female, F1	0	14	138	1,363				
Two generation	Lymphocyte fraction (%)								
F0: exposure started 10 wks prior to mating F1: maternal exposure throughout gestation/lactation; dietary exposure post weaning until necropsy Data quality: ^e High (1.0)	Male, F1 (n = 10)								
	Mean (SD)	88.2 (4.4)	90.9 (2.7)	87.7 (5.9)	87.3 (5.7)				
	% change ^b	–	3%	–1%	–1%				
	Female, F1 (n = 10)								
	Mean (SD)	83.6 (9.4)	76.2 (9.6)	83.6 (8.3)	73 (11.6)				
	% change ^b	–	–9%	0%	–13%				
van der Ven et al. (2009)	Doses (mg/kg-d)								
Rats, Wistar	0	0.1	0.3	1	3	10	30	100	
Diet	Basophil cell count in blood (×10⁹/L)								
One generation	Male, F1 (n = 3–4)**								
F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Only males evaluated	Mean (SD)	0.040 (0.004)	0.072 (0.016)	0.063 (0.026)	0.057 (0.016)	0.045 (0.016)	0.048 (0.028)	0.068 (0.008)	0.035 (0.030)
	% change ^b	–	80%	57%	43%	12%	20%	70%	–12%
	Lymphocyte cell fraction in blood (%)								
Male, F1 (n = 3–4)**									
Mean (SD)	89.64 (0.29)	89.87 (0.26)	89.45 (0.29)	89.72 (0.18)	88.61 (0.4)	89.61 (0.25)	88.65 (0.15)	85.9 (0.23)	
% change ^b	–	0%	0%	0%	–1%	0%	–1%	–4%	
WBC count in blood (×10⁹/L)									
Male, F1 (n = 3–4)**									
Mean (SD)	5.10 (1.01)	7.18 (1.44)	5.72 (1.79)	6.53 (0.72)	4.90 (1.71)	5.92 (2.27)	6.55 (0.14)	4.05 (1.50)	
% change ^b	–	41%	12%	28%	–4%	16%	28%	–21%	

Reference and study design	Results									
Hachisuka et al. (2010) Rats, SD:IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11 Only males evaluated Data quality: ^c Medium (1.9)	Doses (mg/kg-d)^c									
			0	14.8	146.3					1,505
	Activated T cell fraction in blood (%)									
	Male, F1, PNW 3 (n = 10)									
	Mean (SD)	13.51 (3.47)	14.01 (2.16)	11.81 (1.96)					10.40* (2.02)	
	% change ^b	–	4%	–13%					–23%	
	Male, F1, PNW 11 (n = 10)									
	Mean (SD)	1.45 (0.54)	1.35 (0.6)	1.27 (0.47)					1.32 (0.24)	
	% change ^b	–	–7%	–12%					–9%	
	Lymphocyte fraction in blood (%)									
	Male, F1, PNW 3 (n = 9–10)									
	Mean (SD)	78.88 (4.74)	79.02 (3.18)	81.69 (3.81)					81.41 (4.06)	
	% change ^b	–	0%	3%					3%	
	Male, F1, PNW 11 (n = 10)									
	Mean (SD)	84.64 (5.46)	84.27 (4.88)	87.56 (4.33)					86.44 (3.36)	
% change ^b	–	0%	3%					2%		
NK cell fraction in blood (%)										
Male, F1, PNW 3 (n = 10)										
Mean (SD)	0.12 (0.03)	0.1 (0.03)	0.09 (0.02)					0.08* (0.04)		
% change ^b	–	–17%	–25%					–33%		
Male, F1, PNW 11 (n = 10)										
Mean (SD)	0.27 (0.07)	0.23 (0.08)	0.27 (0.07)					0.25 (0.09)		
% change ^b	–	–15%	0%					–7%		
WBC count in blood (×10²/μL)										
Male, F1, PNW 3 (n = 10)										
Mean (SD)	35.3 (11.3)	30.9 (10)	47.5* (11.8)					39.6 (7.9)		
% change ^b	–	–12%	35%					12%		
Male, F1, PNW 11 (n = 10)										
Mean (SD)	82.1 (17.8)	109.8* (30.8)	110* (29.3)					103.4 (34.1)		
% change ^b	–	34%	34%					26%		
Histopathology										
van der Ven et al. (2009) Rats, Wistar Diet One generation F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	Male, F1									
			0	0.1	0.3	1	3	10	30	100
	Female, F1									
	WBC count in bone marrow (×10⁹/L)									
	Male, F1 (n = 3–4)**									
	Mean (SD)	9.3 (3.4)	15.0 (9.3)	17.4 (8.5)	13.0 (3.0)	17.9 (4.2)	20.2 (4.1)	16.3 (5.0)	17.6 (4.8)	
% change ^b	–	61%	87%	40%	92%	117%	75%	89%		
CD161a (NK) subpopulation fraction in spleen (%)										
Male, F1 (n = 3–5)**										
Mean (SD)	7.9 (0.4)	8.8 (0.8)	8.6 (1.4)	8.9 (1.3)	9.6 (0.6)	8.9 (0.8)	9.0 (1.5)	11.3 (1.3)		

Reference and study design	Results								
Data quality: ^c High (1.2)	% change ^a	–	11%	9%	13%	22%	13%	14%	43%
	Splenic marginal zone enlargement (incidence)								
	Male, F1 (n = 8–10)								
	Incidence	1/8	– ^d	– ^d	– ^d	– ^d	– ^d	– ^d	7/10*
Hachisuka et al. (2010) Rats, SD:IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11 Data quality: ^c Medium (1.9)	Doses (mg/kg-d)^c								
	Male, F1		0		15		146		1,505
	Female, F1								
	CD4NKT (NK) cell fraction in spleen (%)								
	Male, F1, PNW 3 (n = 10)								
		Mean (SD)	6.47 (0.61)		6.28 (0.81)		6.4 (1.31)		5.63* (0.81)
		% change ^b	–		–4%		–1%		–13%
	Male, F1, PNW 11 (n = 10)								
		Mean (SD)	12.53 (1.88)		12.89 (1.85)		13.78 (2.66)		13.09 (1.72)
		% change ^b	–		3%		10%		4%
	CD8+ CD4- (cytotoxic T-cell) cell fraction in spleen (%)								
	Male, F1, PNW 3 (n = 10)								
		Mean (SD)	6.86 (0.95)		8.12 (2.16)		6.99 (1.42)		6.43 (1.44)
		% change ^b	–		28%		10%		1%
	Male, F1, PNW 11 (n = 10)								
		Mean (SD)	14.42 (2.23)		18.54* (4.34)		16.85 (4.31)		18.87* (4.82)
		% change ^b	–		29%		17%		31%
	N NKR1A+CD4- (NK) cell fraction in spleen (%)								
	Male, F1, PNW 3 (n = 10)								
		Mean (SD)	5.75 (0.35)		6.06 (1.09)		5.65 (0.87)		5.09* (0.76)
	% change ^b	–		5%		–2%		–11%	
Male, F1, PNW 11 (n = 10)									
	Mean (SD)	10.63 (1.63)		9.97 (3.44)		11.38 (2.47)		9.44 (2.39)	
	% change ^b	–		–6%		7%		–11%	
Activated T-cell fraction in thymus (%)									
Male, F1, PNW 3 (n = 10)									
	Mean (SD)	2.67 (0.87)		2.46 (0.80)		1.82* (0.55)		1.87 (1.15)	
	% change ^b	–		–4%		–29%		–27%	
Male, F1, PNW 11 (n = 10)									
	Mean (SD)	0.92 (0.97)		0.74 (0.51)		1.02 (0.84)		1.04 (0.70)	
	% change ^b	–		–20%		11%		13%	
Increased starry sky appearance in thymus									
Male, F1, PNW 3 (n = 10)									
	Incidence	0/10		0/10		4/10*		1/10	
Male, F1, PNW 11 (n = 10)									
	Incidence	0/10		0/10		0/10		0/10	
Female, F1, PNW 3 (n = 10)									
	Incidence	0/10		0/10		0/10		0/10	

Reference and study design	Results				
	Female, F1, PNW 11 (n = 10)				
	Incidence	0/10	0/10	3/10	0/10
	NK cell fraction in thymus (%)				
	Male, F1, PNW 3 (n = 10)				
	Mean (SD)	0.07 (0.03)	0.07 (0.03)	0.06 (0.02)	0.07 (0.05)
	% change ^b	–	0%	–43%	0%
	Male, F1, PNW 11 (n = 10)				
	Mean (SD)	0.2 (0.04)	0.2 (0.05)	0.25 (0.09)	0.27* (0.08)
	% change ^b	–	0%	25%	35%
	Treg cell fraction in thymus (%)				
	Male, F1, PNW 3 (n = 10)				
	Mean (SD)	7.7 (2.57)	5.15* (0.94)	7.69 (1.27)	7.85 (2.85)
	% change ^b	–	–33%	0%	–5%
	Male, F1, PNW 11 (n = 10)				
Mean (SD)	4.16 (1.09)	3.98 (0.87)	4.41 (0.76)	4.32 (1.22)	
% change ^b	–	–1%	6%	4%	

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.

**Significant dose response trend as reported by study authors.

^aPercent change compared to control calculated as: (treated value – control value)/control value × 100.

^bF1 and F2 offspring doses presented as mean maternal gestational F0 and F1 doses, respectively.

^cTWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PNDs 1–9, and PNDs 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = (8.1 mg/kg-day × 11 days) + (14.3 mg/kg-day × 10 days) + (21.3 mg/kg-day × 12 days)/33 days = 14.8 mg/kg-day.

^dNot measured; only control and high-dose values reported.

^eBased on OPPT data evaluation criteria.

Table 1-12. Evidence pertaining to observational immune system effects in animals following exposure to HBCD as adults

Reference and study design	Results				
<i>Organ weight</i>					
Ema et al. (2008)	Doses (mg/kg-d)				
Rats, CRL:CD(SD)	Male, F0	0	10	101	1,008
Diet	Female, F0	0	14	141	1,363
Two generation	Absolute spleen weight (mg)				
F0: exposure started 10 wks prior to mating	Male, F0 (n = 22–24)				
F1: dietary exposure post weaning until necropsy	Mean (SD)	848 (136)	828 (109)	855 (160)	843 (248)
	% change ^a	–	–2%	1%	–1%
	Female, F0 (n = 17–24)				
	Mean (SD)	588 (75)	577 (83)	570 (89)	584 (72)
	% change ^a	–	–2%	–3%	–1%

Reference and study design	Results								
F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Data quality:^b High (1.0)	Absolute thymus weight (mg)								
	Male, F0 (n = 22–24)								
	Mean (SD)	323 (88)		305 (82)		299 (64)		315 (71)	
	% change ^a	–		–6%		–7%		–2%	
	Female, F0 (n = 17–24)								
Mean (SD)	232 (38)		238 (63)		252 (73)		200 (64)		
% change ^a	–		3%		9%		–14%		
van der Ven et al. (2006) Rats, Wistar Gavage 28-d exposure starting on PNW 11 Data quality:^b High (1.3)	Doses (mg/kg-d)								
	0	0.3	1	3	10	30	100	200	
Absolute spleen weight (g)									
Male (n = 4–5)									
Mean (SD)	0.51 (0.09)	0.59 (0.13)	0.78 (0.55)	0.52 (0.05)	0.58 (0.08)	0.47 (0.03)	0.49 (0.05)	0.50 (0.10)	
% change ^a	–	16%	53%	2%	14%	–8%	–4%	–2%	
Female (n = 4–5)**									
Mean (SD)	0.41 (0.04)	0.37 (0.04)	0.38 (0.06)	0.44 (0.01)	0.40 (0.04)	0.49 (0.08)	0.53 (0.04)	0.37 (0.05)	
% change ^a	–	–10%	–7%	7%	–2%	20%	29%	–10%	
Absolute thymus weight (g)									
Male (n = 4–5)**									
Mean (SD)	0.47 (0.08)	0.45 (0.08)	0.52 (0.17)	0.47 (0.07)	0.50 (0.09)	0.37 (0.06)	0.42 (0.09)	0.38 (0.13)	
% change ^a	–	–4%	11%	0%	6%	–21%	–11%	–19%	
Female (n = 4–5)									
Mean (SD)	0.42 (0.06)	0.28 (0.10)	0.36 (0.09)	0.35 (0.07)	0.44 (0.07)	0.43 (0.08)	0.42 (0.08)	0.37 (0.10)	
% change ^a	–	–33%	–14%	–17%	5%	2%	0%	–12%	
Hematology									
Ema et al. (2008) Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: maternal exposure throughout gestation/lactation; dietary exposure post weaning until necropsy Data quality:^b High (1.0)	Doses (mg/kg-d)								
	Male, F0								
		0		10		101		1,008	
	Female, F0								
		0		14		141		1,363	
	Lymphocyte fraction (%)								
	Male, F0 (n = 10)								
	Response	88.5 (6.5)		88.8 (2.4)		88.8 (3.9)		87.5 (4.6)	
	% change ^a	–		0%		0%		–1%	
	Female, F0 (n = 10)								
Mean (SD)	72.5 (8.7)		85* (5)		78.4 (9.5)		70.8 (9)		
% change ^a	–		17%		8%		–2%		
Segmented neutrophil fraction (%)									
Male, F0 (n = 10)									
Mean (SD)	8.00 (5.24)		8.24 (1.98)		7.68 (3.26)		8.68 (4.61)		
% change ^a	–		3%		–4%		8%		
Female, F0 (n = 10)									

Reference and study design	Results								
		Mean (SD)	21.68 (8.08)	10.56* (4.19)	16.84 (9.19)	23.28 (8.13)			
	% change ^a	–	–51%	–22%	7%				
	Stab form neutrophil fraction (%)								
	Male, F0 (n = 10)								
	Mean (SD)	0.48 (0.73)	0.36 (0.3)	0.64 (0.28)	0.56 (0.51)				
	% change ^a	–	–25%	33%	17%				
	Female, F0 (n = 10)								
	Mean (SD)	1.32 (0.57)	0.60* (0.39)	0.84 (0.55)	1.12 (0.7)				
	% change ^a	–	–55%	–36%	–15%				
van der Ven et al. (2006)	Doses (mg/kg-d)								
Rats, Wistar	Male	0	0.3	1	3	10	30	100	200
Gavage	Lymphocyte cell fraction in blood (%)								
28-d exposure starting on PNW 11	Male (n = 3–5)								
	Mean (SD)	89.1 (2.5)	89.0 (3.7)	85.4 (5.9)	85.3 (2.0)	86.7 (3.7)	88.9 (3.8)	84.2 (8.1)	88.1 (3.1)
Data quality:^b	% change ^a	–	0%	–4%	–4%	–3%	0%	–5%	–1%
High (1.3)									
<i>Histopathology</i>									
van der Ven et al. (2006)	Doses (mg/kg-d)								
Rats, Wistar	0	0.3	1	3	10	30	100	200	
Gavage	CD4 (Th) cells per spleen (cells ×10⁷)								
28-d exposure starting on PNW 11	Male (n=1–5)**								
	Mean (SD)	14.0 (4.7)	15.2 (n/a)	13.3 (4.8)	11.4 (n/a)	10.5 (0.9)	9.0 (n/a)	11.2 (n/a)	10.0 (2.0)
Data quality:^b	% change ^a	–	9%	–5%	–19%	–25%	–36%	–20%	–29%
High (1.3)	Total immune cells per spleen (cells ×10⁷)								
	Male (n=1–5)**								
	Mean (SD)	48.7 (10.5)	49.6 (n/a)	47.1 (15.4)	44.4 (n/a)	39.4 (3.8)	29.7 (n/a)	37.0 (n/a)	35.8 (1.1)
	% change ^a	–	2%	–3%	–9%	–19%	–39%	–24%	–26%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.

**Significant dose response trend as reported by study authors.

^aPercent change compared to control calculated as: (treated value – control value)/control value × 100

^bBased on OPPT data evaluation criteria.

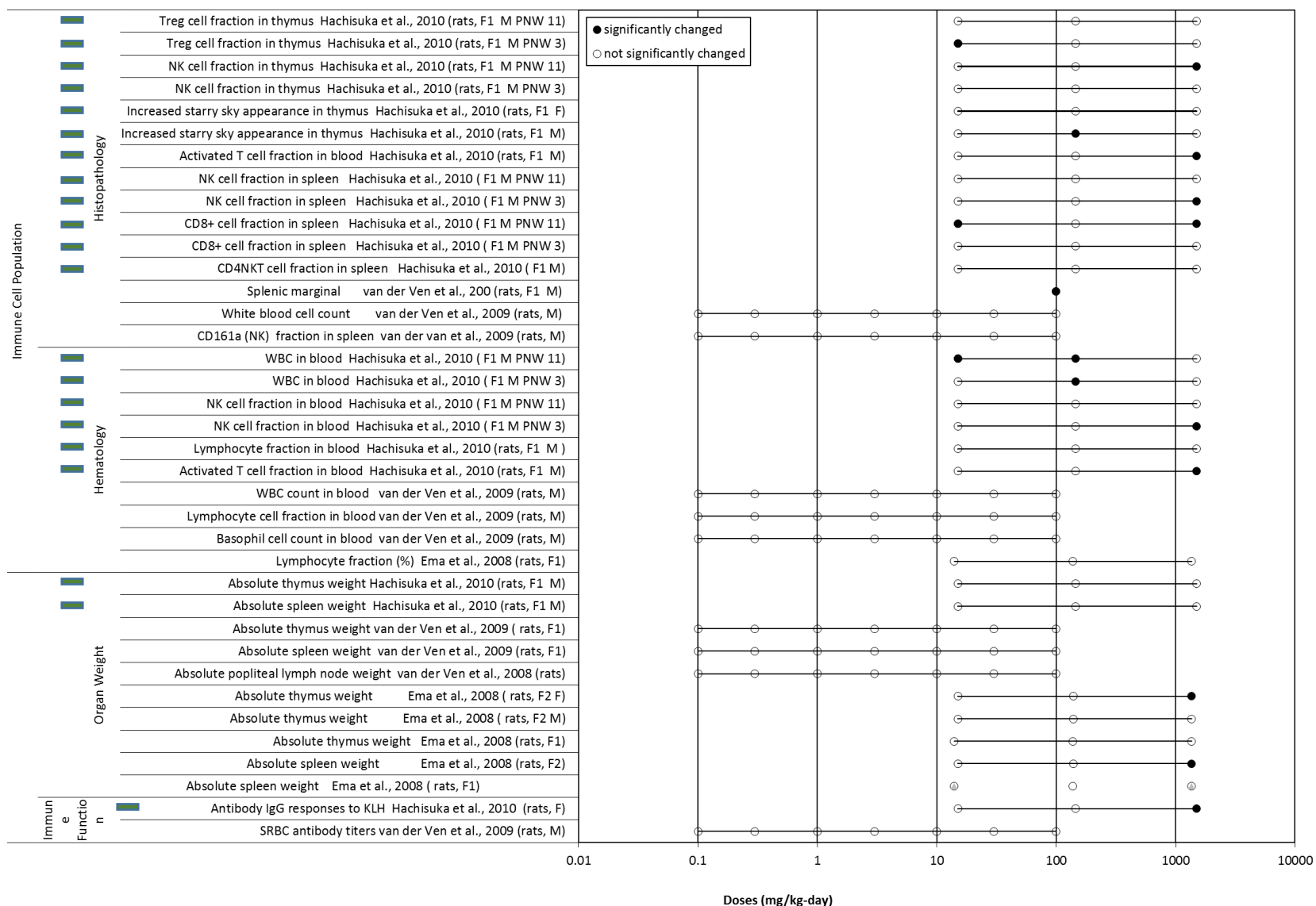


Figure 1-9. Exposure response array of immune system following oral exposure. Most data was from [Hachisuka et al. \(2010\)](#), which scored a Medium in data quality evaluation (indicated with ■). All other studies scored a High.

1.6.3 Mechanistic Evidence

Mechanistic information to support HBCD-mediated effects on the immune system is limited. Several recent in vitro studies in human immune cells suggest that HBCD may alter immune function through activation of MAPK signaling pathways (ERK1/2 and p38) resulting in increased secretion of IFN γ and IL-1 β , pro-inflammatory cytokines that regulate immune function ([Almughamsi and Whalen, 2016](#); [Anisuzzaman and Whalen, 2016](#); [Canbaz et al., 2016a](#)). Similarly, pro-inflammatory effects driven by were observed in human bronchial epithelial cells (BEAS-2B); HBCD exposure increased expression of proinflammatory cytokines (IL-6 and IL-8) and ICAM-1, a cell surface marker often expressed by immune cells, which were mediated by activation of MAPK signaling pathways ([Koike et al., 2016](#)). One study using human monocyte-derived dendritic cells found that co-exposure with HBCD enhanced IL-6 and IL-8 secretion elicited by environmental allergens ([Canbaz et al., 2016a](#)).

[Koike et al. \(2012\)](#) used bone marrow-derived dendritic cells prepared from atopic-prone NC/Nga mice to investigate HBCD effects on the immune response in vitro. HBCD (10 $\mu\text{g}/\text{mL}$) increased cell proliferation and expression of a dendritic activation marker, DEC205. Bone marrow-derived dendritic cells differentiated in the presence of HBCD also showed enhanced MHC class II, CD80, CD86, and CD11c expression. These in vitro data are supported by two studies using the guinea pig maximization test method that indicated that HBCD may act as a mild skin allergen ([Nakamura et al., 1994](#); [Momma et al., 1993](#)). Taken together, these studies suggest that HBCD may stimulate an immune response by increasing the activity of antigen-presenting cells. In vitro, HBCD altered several aspects of human NK cell function, including decreased target cell binding, expression of surface binding proteins, lytic function, and ATP levels ([Hinkson and Whalen, 2010, 2009](#)); however, in vivo NK cell activity was unaffected in rats ([van der Ven et al., 2009](#); [van der Ven et al., 2006](#)).

1.7 Genotoxicity

A limited number of studies have investigated the genotoxicity of HBCD; these are summarized in Table 1-13. The majority of these studies were standard Ames tests for detecting mutagenic potential in *Salmonella typhimurium*. These tests, which employ different strains of bacteria that have been developed with pre-existing mutations, including *S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538, are referred to as reversion assays ([Maron and Ames, 1983](#)). Most of these assays conducted with HBCD yielded negative results ([Huntingdon Research, 1990](#); [International, 1990](#); [Labs, 1990](#); [Litton, 1990](#); [Pharmakologisches, 1990](#); [Zeiger et al., 1987](#)). Among the few assays performed to determine the genotoxicity of HBCD in prokaryotic systems, one in yeast ([Litton, 1990](#)), one detecting chromosomal aberrations in human peripheral lymphocytes in vitro ([Microbiological, 1996](#)), and one in vivo mouse micronucleus test following intraperitoneal (i.p.) injections of HBCD ([BASF, 2000](#)) were negative, even when tested at cytotoxic concentrations.

Table 1-13. Summary of genotoxicity studies of HBCD

Test/species/strain/ route	Test doses (per plate) ^a	Results ^b		Notes	Reference
		-S9	+S9		
Eukaryotic systems, in vitro					
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	50–5,000 µg (HBCD bottoms) in acetone	+	+	No cytotoxicity observed. Dose- response observed in TA1535 (-S9) ≥100 µg/plate. TA100 positive at highest dose only (5,000 µg/plate). All doses had a black precipitate thought to be carbon.	Ethyl (1990b)
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	50 µg (421–32B) (solvent not reported)	–	–		Litton (1990)
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	2–1,000 µg (GLS-S6-41A) in DMSO	–	–		Gsri (1978)
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	100–10,000 µg in DMSO	–	–	Doses ≥1,000 µg were insoluble.	Zeiger et al. (1987)
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	250 µg (Firemaster, FM-100, Lot 53, white powder) in DMSO	–	–	Doses ≥250 µg were insoluble.	Labs (1990)
	1,000 µg (FM-100, Lot 3322, liquid residue) in DMSO	–	+	Significant in TA1535 at highest dose only.	
<i>S. typhimurium</i> TA98, TA100, TA1537	3,000 µg in DMSO	–	–	Doses ≥1,000 µg were partially insoluble.	Pharmakologisches (1990)
<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	5,000 µg in DMSO	–	–	No cytotoxicity observed.	International (1990)

Test/species/strain/ route	Test doses (per plate) ^a	Results ^b		Notes	Reference
		-S9	+S9		
<i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, TA1537	10,000 µg (Pyroguard SR-103) in DMSO	-	-		Ogaswara and Hanafusa (1993)
<i>S. typhimurium</i> TA98, TA100, TA1535	10,000 µg in DMSO	-	-	Insoluble at 10,000 µg.	Huntingdon Research (1990)
Prokaryotic non-mammalian systems, in vitro					
<i>Saccharomyces cerevisiae</i> D4	50 µg (solvent not reported)	-	-		Litton (1990)
Mammalian systems, in vivo					
Micronucleus test mouse/NMRI/i.p. injection	2,000 mg/kg in DMSO	- (T)	NA	Toxicity evident as a slight inhibition of erythropoiesis at 2,000 mg/kg. Number of polychromatic erythrocytes with micronuclei from femoral bones evaluated 24 hrs after 2 nd injection.	BASF (2000)
Mammalian systems, in vitro					
Chromosomal aberration test Human peripheral blood lymphocytes	750 µg/mL (-S9) 250 µg/mL (+S9) in DMSO	- (T)	- (T)	Doses 750–2,500 µg/mL were partially insoluble, and fully insoluble >2,500 µg/mL. Repeated test for two harvest time points: 20-hr (-S9) or 4-hr (+S9) incubations, and 20- or 44-hr incubations (-S9 and +S9).	Microbiological (1996)
Reversion assay CHO/V79/Sp5 and SPD8 Intragenic recombination at <i>hprt</i> locus in Sp5 (non-HR) and SPD8 (HR) duplication cell lines	3–20 µg/mL in DMSO	+	NA	A statistically significant, dose-dependent increase in reversion frequency was observed in both assays as determined by linear regression analysis. Significant inhibition of cloning efficiency	Helleday et al. (1999)

Test/species/strain/ route	Test doses (per plate) ^a	Results ^b		Notes	Reference
		-S9	+S9		
				occurred at doses ≥ 15 $\mu\text{g}/\text{mL}$ in the SPD8 assay and ≥ 20 $\mu\text{g}/\text{mL}$ in the Sp5 assay. Cytotoxicity (IC_{50}) measured at 0.02–0.03 mM.	
Unscheduled DNA synthesis rat/F344 male/primary hepatocytes	10 $\mu\text{g}/\text{well}$ in acetone (HBCD bottoms)	+	NA	Five highest doses (from 5 $\mu\text{g}/\text{well}$) showed an increased response with dose over solvent control, but only four highest were statistically significant (χ^2). Highest dose (1,000 $\mu\text{g}/\text{well}$) was cytotoxic.	Ethyl (1990a)

^aLowest effective dose for positive results; highest dose tested for negative results.

^b+ = positive; \pm = equivocal or weakly positive; - = negative; T = cytotoxicity; NA = not applicable.

DMSO = dimethyl sulfoxide

Some positive results have been reported. *S. typhimurium* strain TA1535 was positive for reverse mutations at the highest dose only using a liquid residue of HBCD in DMSO ([Labs, 1990](#)), and strain TA100 was positive also at the highest dose using an unidentified mixture characterized only as HBCD bottoms in acetone ([Ethyl, 1990b](#)). In this same study, TA1535 was positive at ≥ 100 $\mu\text{g}/\text{plate}$ without addition of an S9 microsomal fraction ([Ethyl, 1990b](#)). The number of revertants increased with dose. This was the only Ames study to report dissolving the test article in a solvent other than DMSO (in this case, acetone). DMSO is a free-radical scavenger and can potentially obscure genetic damage due to oxidative radicals. Both strains TA1535 and TA100 were designed to be sensitive to detecting reversions by base substitution, a type of genetic lesion that can result from oxidative DNA damage due to reactive oxygen species (ROS). However, there is only limited evidence in the literature indicating that HBCD exposure may induce oxidative stress ([An et al., 2013](#); [Hu et al., 2009b](#)).

In mammalian systems, a reverse mutation assay with Chinese hamster ovary (CHO) Sp5 and SPD8 cell lines exposed to HBCD ([Helleday et al., 1999](#)) yielded positive results. These two clones exhibit a partial duplication of the hprt gene, causing lethality unless a reversion occurs, either via homologous recombination (SPD8) or non-homologous recombination (Sp5). A statistically significant, dose-dependent increase in reversion frequency was observed in both clones, although at higher doses, there was a significant inhibition of cloning efficiency. In addition, a test of unscheduled DNA synthesis with rat hepatocytes exposed to HBCD bottoms was positive ([Ethyl, 1990a](#)), and also showed an increase in response with dose.

It is noteworthy that in these three studies ([Helleday et al., 1999](#)), the positive results were dose-dependent, observed at nontoxic doses, and in two assays, specific for detecting mutations. However, the Ames tests in the same strains that showed positive results (TA1535 and TA100) were negative in seven other studies, and the results in the reverse mutation assay in CHO cells ([Helleday et al., 1999](#)) have not been confirmed by another group.

2 DOSE-RESPONSE ANALYSIS

2.1 Supplemental Information on Non-Cancer Dose Response Analysis

2.1.1 Additional Considerations for Selection of Studies for Dose-Response Analysis

As discussed in Section 1.3.2, studies in humans were not adequate to support conclusions regarding the relationship between HBCD exposure and effects on the thyroid, male reproduction, or nervous system, and accordingly do not support dose-response analysis. In the absence of adequate human data, animal toxicity studies were used for dose-response analysis. Studies in animals provided evidence of thyroid toxicity, liver toxicity, female reproductive, and developmental toxicity following oral exposure to hexabromocyclododecane (HBCD). These hazards have been carried forward for dose-response analysis. While there is also evidence to support nervous system toxicity following exposure to HBCD during development in animal studies, these data sets were not carried forward for dose-response analysis. Likewise, data sets for male reproductive effects, adult neurological effects, immune system effects, genotoxicity, and cancer were not carried forward for dose-response analysis. For a complete discussion, see Section 1.3.2.

The effects determined to best represent each of the hazards were identified in Section 1.3.2, and studies that evaluated these effects are considered in this section for dose-response analysis. In order to identify the stronger studies for dose-response analysis, several attributes of the studies were reviewed. Preference was given to studies using designs reasonably expected to detect a dose-related response. Chronic or subchronic toxicity studies are necessary for estimating risks related to chronic or subchronic exposures under the conditions of use within the scope of the TSCA risk evaluation. Studies with a broad exposure range and multiple exposure levels are preferred to the extent that they can provide information about the shape of the exposure-response relationship. Additionally, with respect to measurement of the endpoint, studies that can reliably measure the magnitude and/or degree of severity of the effect are preferred.

Experimental animal studies considered for each hazard and effect were evaluated using general study quality considerations discussed above and in the Systematic Review Methods section. The rationales for selecting the strongest studies to represent these hazards are summarized below.

2.1.1.1 Thyroid Effects

Regulation of thyroid hormones is complex and homeostasis is largely maintained via HPT axis feedback mechanisms. Reductions in serum T3 or T4 triggers release of TSH from the pituitary, which stimulates the thyroid gland to increase secretion of T3 and T4 stores from the colloid ([Fisher and Nelson, 2012](#)). Decreased T4 is expected to be the primary driver of HBCD-

mediated thyroid effects that triggers release of TSH. Indeed, this is supported by mechanistic studies that indicate that that observed decreases in T4 may be largely driven by hepatic induction of enzymes that metabolize this hormone (See Section 1.1.6, Mechanistic Evidence).

Despite demonstrating a sensitive response to HBCD exposure, follicle size was not selected for modeling because: (1) quantitative data for follicle size changes were provided only in one study (Ema, 2008); (2) although this is generally a well conducted study, details of the methods of analysis (e.g., the criteria used to determine whether an animal showed decreased follicle size) were not provided; and (3) although changes in thyroid histopathology (e.g., follicle size, epithelial cell hypertrophy) can be useful indicators of changes in thyroid function/homeostasis, they are less direct measures of thyroid toxicity and it would be difficult to determine an appropriate benchmark response (BMR).

Serum thyroxine (T4) was selected for dose-response analysis of thyroid effects (see Section 1.3.2). Three studies in rats reported treatment-related decreases in serum T4 following oral exposure ([Ema et al., 2008](#); [van der Ven et al., 2006](#); [WIL Research, 2001](#)). Table 2-1 provides an overview of the study designs for those studies reporting T4 levels that were evaluated for dose-response analysis.

[Ema et al. \(2008\)](#) reported a decrease in serum T4 levels in both male and female rats from the F0 (30 and 31% at the high dose, respectively) and F1 (10 and 28% at the high dose, respectively) generations. [van der Ven et al. \(2006\)](#) reported similar effects on serum T4 (26% reduction at the high dose) in adult female rats exposed for 28 days. [WIL Research \(2001\)](#) reported changes in T4 levels in rats exposed to HBCD for 90 days, but inadequate reporting of thyroid hormone measurement methods, high proportion (50%) of samples below the limit of detection, and unusually low control thyroid-stimulating hormone (TSH) levels reduced the confidence in these results, bringing into question the conduct of the assays.

2.1.1.2 Liver Effects

The most consistently observed liver outcome was liver weight changes. Dose-related increases were consistently observed across species, sexes, and age from multiple studies of various designs and exposure durations ([Yanagisawa et al., 2014](#); [Maranghi et al., 2013](#); [Saegusa et al., 2009](#); [Ema et al., 2008](#); [WIL Research, 2001, 1997](#)). Limited support for HBCD effects on the liver are provided by histopathological examination. A subset of the rat studies ([Saegusa et al., 2009](#); [WIL Research, 2001, 1997](#)) and one mouse study ([Maranghi et al., 2013](#)) reported increased vacuolation (generally of minimal to mild severity) in HBCD-exposed animals, but these responses were not dose-related. The content of the vacuoles was investigated only by [WIL Research \(2001\)](#) and characterized as lipid. Other histological findings were less frequently observed and included some additional evidence of fatty change (steatosis) ([Yanagisawa et al., 2014](#)), hypertrophy ([Yanagisawa et al., 2014](#); [WIL Research, 1997](#)), and inflammation ([Maranghi et al., 2013](#)). Statistically or biologically significant elevations in serum liver enzymes were not associated with HBCD exposure in rats or mice in multiple studies ([Yanagisawa et al., 2014](#); [WIL Research, 2001, 1997](#)), however in contrast mechanistic evidence *in vitro* suggests that HBCD may in fact induce hepatic microsomal enzymes ([Crump et al., 2010](#); [Crump et al., 2008](#); [Germer et al., 2006](#)). Microsomal enzyme induction is a proposed key event in initiating the perturbation of the HPT axis that leads to reduced T4 levels. Given limited evidence of HBCD-related histopathological changes and no clear evidence of clinical chemistry changes,

the biological significance of liver weight changes is unclear. While increased liver weight was not consistently associated with other toxicological evidence of liver toxicity in rodents given a standard diet, biochemical and histopathological effects indicative of steatosis were observed in mice fed a high-fat diet ([Yanagisawa et al., 2014](#)). A high-fat diet may therefore represent a susceptibility factor for TCE toxicity ([Bernhard et al., 2016](#)).

Increased liver weight was selected for dose-response analysis of liver effects (see Section 1.3.2). This endpoint was reported in six studies in rats ([Saegusa et al., 2009](#); [Ema et al., 2008](#); [van der Ven et al., 2006](#); [WIL Research, 2001, 1997](#)) and mice ([Maranghi et al., 2013](#)). The developmental study by [Saegusa et al. \(2009\)](#) and the 28-day study by [WIL Research \(1997\)](#) used similar dose ranges as the longer-duration studies ([Ema et al., 2008](#); [WIL Research, 2001](#)) and observed similar findings in pup or adult liver weights. A significant trend in increased liver weight was reported by [van der Ven et al. \(2006\)](#) following a 28-day adult exposure at lower doses, but in female rats only. Data from these shorter exposure duration studies were not used for dose-response analysis because similar effects were observed in the studies with longer exposure durations ([Ema et al., 2008](#); [WIL Research, 2001](#)) that better reflect effects expected following subchronic or chronic exposure. Similarly, [Maranghi et al. \(2013\)](#) was not used for dose-response analysis because it used a relatively short (28-day) exposure and a single dose group that is less informative for evaluating a dose-response relationship.

2.1.1.3 Female Reproductive Effects

See the Risk Evaluation document ([EPA-HQ-OPPT-2016-0735](#)) for details on this endpoint.

2.1.1.4 Developmental Effects

Several studies in animals exposed during gestation and lactation provide some evidence of developmental effects associated with HBCD, including reduced offspring viability ([Ema et al., 2008](#)), decreased pup body weight ([Maranghi et al., 2013](#); [Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#)), altered development of the skeletal system, and delayed eye opening ([Ema et al., 2008](#)). The strongest evidence of developmental effects is based on findings of reduced offspring viability and decreased pup body weight. Reduced viability was observed in the two-generation study by [Ema et al. \(2008\)](#); the decreases in viability were dose-related and observed on both PND 4 and 21. Effects were seen only in F2 offspring. This is consistent with decreased viability manifesting after multigenerational exposure, although that hypothesis cannot be established based on the current developmental literature for HBCD (i.e., a single two-generation study). Effects on pup body weight were demonstrated in several studies in rats using different strains and exposure durations ([Saegusa et al., 2009](#); [van der Ven et al., 2009](#); [Ema et al., 2008](#)). Other developmental effects, including changes in bone development and delayed eye opening, were only reported in a single study and with a less clear dose-response relationship ([van der Ven et al., 2009](#); [Ema et al., 2008](#)). Therefore, pup body weight and viability were selected for dose-response analysis of developmental effects.

[Ema et al. \(2008\)](#) evaluated changes in pup body weight in rats that were continuously exposed to HBCD across two generations. Treatment-related effects on pup body weight were measured throughout early postnatal development (PNDs 0, 4, 7, 14, and 21) in three dose groups, covering a dose range of approximately 2.5 orders of magnitude. This study used an adequate sample size (n = 13–24) and litter as the statistical unit. [Maranghi et al. \(2013\)](#) was considered less appropriate to support derivation of an RfD because the study used only one dose group, which

is less informative for evaluating dose-response relationships, and a relatively short exposure duration (28 days). [van der Ven et al. \(2009\)](#) used a dose range that was >10-fold lower than those used in the [Ema et al. \(2008\)](#) and [Saegusa et al. \(2009\)](#) studies and, in general, did not show a clear pattern of dose-related changes in pup body weight on different days of lactation.

2.1.2 BMR Selection

A set of dose-response models that are consistent with a variety of potentially underlying biological processes were applied to empirically model the dose-response relationship in the range of the observed data. The models in EPA's Benchmark Dose Software (BMDS, version 2.6) were applied. Consistent with EPA's *Benchmark Dose Technical Guidance Document* ([U.S., 2012](#)), the benchmark dose (BMD) and 95% lower confidence limit on the BMD (BMDL) were estimated using a benchmark response (BMR) to represent a minimal, biologically significant level of change, described here as relative deviation (RD). In the absence of information regarding the level of change that is considered biologically significant, a BMR of 1 standard deviation (SD) from the control mean for continuous data or a BMR of 10% extra risk (ER) for dichotomous data is used to estimate the BMD and BMDL, and to facilitate a consistent basis of comparison across endpoints, studies, and assessments. Endpoint-specific BMRs are described further below. Where modeling was feasible, the estimated BMDLs were used as points of departure (PODs). Further details, including the modeling output and graphical results for the model selected for each endpoint, can be found in Section 3.2. Where dose-response modeling was not feasible, NOAELs or LOAELs were identified and are summarized in Table 2-4.

2.1.2.1 Thyroid Effects

Changes in T4 levels described by [Ema et al. \(2008\)](#) were amenable to BMD modeling. In selecting a BMR (i.e., a change in T4 levels considered biologically significant), pregnant females and their offspring were addressed separately from adult males. Early life development is generally recognized as being particularly sensitive to thyroid perturbation. Thyroid hormones play a critical role in coordinating complex developmental processes, and perturbations of thyroid hormone levels in a pregnant woman or neonate can have persistent adverse health effects for the child. During early gestation, the developing fetus relies solely on thyroid hormones of maternal origin. As the fetus begins to produce thyroid hormones, there is less reliance on maternal thyroid hormones; however, early development remains a sensitive life stage for hormone deficits, largely due to minimal reserve capacity when compared to adults ([Gilbert and Zoeller, 2010](#)).

Reductions in maternal T4 during pregnancy or the early postnatal period are strongly associated with adverse neurological outcomes in offspring. In humans, mild to moderate maternal thyroid insufficiency is associated with higher risk for persistent cognitive and behavioral deficits in children. In general, mild to moderate thyroid insufficiency in pregnant women was defined as serum T4 levels below the 10th percentile for the study population, which is associated with a 15–30% decrease relative to the corresponding median ([Finken et al., 2013](#); [Julvez et al., 2013](#); [Román et al., 2013](#); [Henrichs et al., 2010](#); [Haddow et al., 1999](#)). Similar effects have been described in animal studies, with modest reductions in maternal T4 during gestation resulting in behavioral alterations, learning deficits, and neuroanatomical changes in offspring ([Gilbert et al., 2014](#); [Gilbert et al., 2013](#); [Gilbert, 2011](#); [Liu et al., 2010](#); [Ausó et al., 2004](#)). Thyroid inhibition during gestation and lactation that resulted in drops in mean maternal T4 levels of ~10–17% have been found to elicit neurodevelopmental toxicity in offspring ([Gilbert et al., 2016](#); [Gilbert,](#)

[2011](#)). Although there are some differences in HPT regulation (e.g., serum hormone binding proteins, hormone turnover rates, and timing of in utero thyroid development), rodents are generally considered to be a good model for evaluating the potential for thyroid effects of chemicals in humans ([Zoeller et al., 2007](#)), although a National Academies of Sciences review of the iodide uptake inhibitor perchlorate ([NRC, 2005](#)) concluded that there may be quantitative differences. Based on the overall data observed in both humans and animals, a BMR of 10% RD from control mean was determined to be a minimally biologically significant degree of change when performing BMD modeling using female rat data.

The available thyroid literature does not support identification of a biologically significant change in T4 levels in adult males as decreases in T4, and more generally thyroid function, have not been conclusively linked to similarly severe outcomes as in females. Nevertheless, males with depressed T4 values are part of the subpopulation that experiences thyroid dysfunction. Selecting a biologically-based BMR is also complicated by the inherent variability of thyroid hormones. Individuals show relatively narrow variability around a set point; however, set points can vary considerably between individuals, resulting in a broad population range that is considered normal ([Andersen et al., 2002](#)). Thus, it is possible for an individual to have thyroid levels that fall within the normal population range, but are abnormal relative to their homeostatic set point. Consistent with EPA's *Benchmark Dose Technical Guidance Document* ([U.S., 2012](#)), a BMR of one control SD change from the control mean was applied in modeling T4 data from male rats in the absence of a biological basis for selecting a BMR.

Additionally, a BMR of 10% RD from control means, supported by the literature on the effects of thyroid insufficiency in pregnant females and their offspring, was applied in modeling the male T4 data. In looking across the available HBCD studies, there does not appear to be a strong sex-specific effect on T4 responses (see Table 1-3). Differences in dose-response (i.e., similar responses at the high dose but divergent responses at the lower doses) was observed in the F0 male and female data sets that were modeled ([Ema et al., 2008](#)). These differences likely reflect the inherent variability of thyroid hormones within a population, especially for a relatively small sample size as used in [Ema et al. \(2008\)](#), and not a sex-specific difference in response. Under the assumption that differences in thyroid hormone response in male and female rats exposed to HBCD are not sex-specific but rather a reflection of hormone variability, using a BMR of 10% RD was considered reasonable.

2.1.2.2 Liver Effects

See the Risk Evaluation document ([EPA-HQ-OPPT-2016-0735](#)) for details on this endpoint.

2.1.2.3 Female Reproductive Effects

2.1.2.3.1 Primordial Follicle Count

Decreased primordial follicle count as reported in the two-generation reproductive toxicity study by [Ema et al. \(2008\)](#) was amenable to BMD modeling. Because primordial follicles are formed during gestation, the average dose during this critical window was used for BMD modeling. A BMR of 10% RD from control levels was applied in modeling this endpoint under the assumption that it represents a minimal biologically significant effect. There is no consensus in the scientific community regarding the degree of change that is considered to be adverse. In this situation, it has been suggested that a detectable decrease in follicle number should be considered

adverse ([Heindel, 1998](#)). Power analyses by [Heindel \(1998\)](#) focused on identifying follicle counts reduced by $\geq 20\%$, suggesting that a reduction of this magnitude is considered a critical effect level. Thus, a 10% reduction was selected to represent a minimally important degree of change.

2.1.2.3.2 Pregnancy Incidence

In the study by [Ema et al. \(2008\)](#), the increased incidence of non-pregnancy in HBCD-exposed F0 or F1 rats alone was not statistically significant with either pairwise test (as reported by authors) or Cochran-Armitage trend test (conducted by EPA). Dose-response curves were shallow and never reached a high response percentage. To increase statistical power and obtain a more precise estimate of the BMD and BMDL, consideration was given to combining F0 and F1 datasets. Cochran-Mantel-Haenszel statistics on F0 and F1 data stratified by dose groups were not significant ($p = 0.59$, $\alpha = 0.05$), indicating no statistical association between generation and response after adjusting for dose. Equality of responses in F0 and F1 rats was also not rejected ($p > 0.2$, $\alpha = 0.05$) by the Breslow-Day test for homogeneity of the odds ratios, and their background response percentages were not detectably different (Fisher's exact, $p = 1.00$). The results of these statistical tests suggested that F0 and F1 datasets were compatible for combining. A statistically significant trend ($p = 0.02$) was found using the Cochran-Armitage test applied to the combined data. The Log-logistic model was selected after dropping the highest dose (see Supplemental Information, Appendix D, Section D.2). F0 and F1 data were also modeled separately after dropping the highest dose. A Likelihood ratio test ($\alpha = 0.05$, d.f. = 3) could not reject equality of the three Log-logistic models from combined dataset and F0, F1 alone. Therefore, the Log-logistic model from the combined dataset was used to derive the BMD and BMDL for increased incidence of non-pregnancy with increasing dose.

A BMR of 5% ER was applied in modeling this endpoint under the assumption that it represents a minimal biologically significant degree of change. Selection of a BMR took into consideration the limited sensitivity of rodent species to effects on fertility and pregnancy outcomes ([U.S., 1996](#)). As noted in [U.S \(1996\)](#), the limited sensitivity of fertility measures in rodents suggests that a POD (i.e., NOAEL, LOAEL, or BMD) based on fertility may not reflect completely the extent of effects on reproduction, such that the BMD may need to be adjusted to reflect that additional uncertainty. Rather than applying an additional uncertainty factor to the POD based on reduced fertility in rats, a BMR of 5%, rather than 10%, was selected. A BMR of 5% ER was also consistent with the functional severity of the endpoint (i.e., reduced fertility).

2.1.2.4 Developmental Effects

2.1.2.4.1 Offspring Loss

Increased offspring loss in the F2 generation from the [Ema et al. \(2008\)](#) study was amenable to BMD nested modeling, using individual animal data obtained from the study authors (personal communication) ([Makris et al., 2016](#)). Two datasets were modeled: offspring loss from implantation through PND 4 and offspring loss from PND 4 (post-culling) through PND 21. Maternal gestational doses (10, 100, and 995 mg/kg-day) were used to model the offspring loss from the implantation through PND 4 dataset because they are reflective of the majority of the exposure window being modeled (i.e., 3 weeks of gestation compared to 4 days of lactation) and early lactational doses are closer to the gestational doses than the average dose during the entire lactational period. For similar reasons, modeling for the PND 4 post-culling through PND 21

dataset was performed using the maternal lactational doses (20, 179, and 1,724 mg/kg-day). Use of maternal lactational doses for modeling the PND 4 to 21 dataset was also consistent with total litter loss in eight high-dose dams that occurred at time points across the lactational period (specifically, PNDs 4, 5, 7, 9, 11, 13, and 18).

The use of a 1% ER BMR for offspring loss as reported in [Ema et al. \(2008\)](#) resulted in $BMDL_{01}$ values for loss from implantation through PND 4 and for offspring loss from PND 4 post-culling through PND 21 in F2 rats that fell in the region of the dose-response curve where the response in dosed animals was similar to the response in the controls (see Figure 2-1).

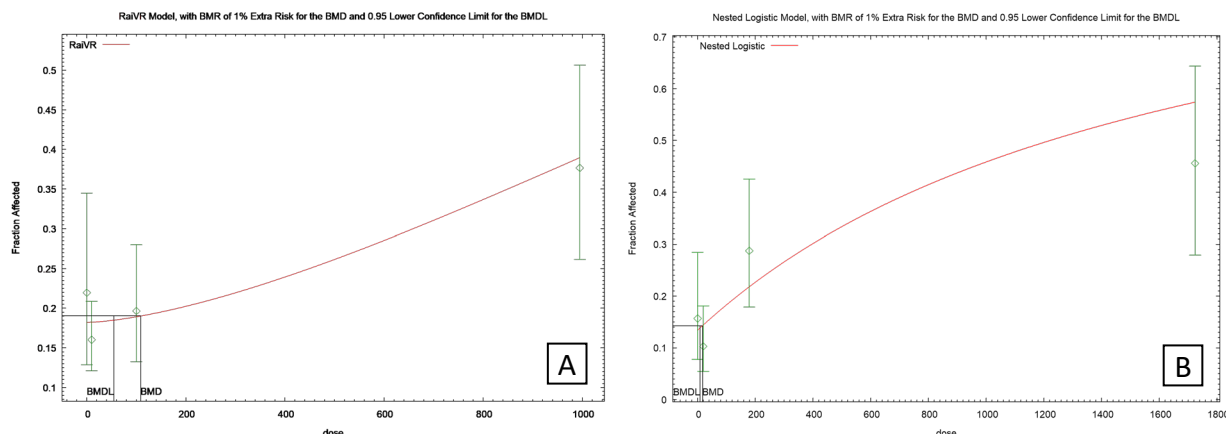


Figure 2-1. BMD modeling plots of incidence of offspring loss from implantation through PND 4 in F2 offspring rats (A) and incidence of offspring loss from PND 4 post-culling through PND 21 in F2 offspring rats (B) from [Ema et al. \(2008\)](#); BMR = 1% ER (see Appendix D, Figures D-31 and D-33).

A NOAEL was also considered as the POD in addition to the POD derived using a BMD modeling approach. As shown in Figure 2-1, there is variation around the response at each dose. Although the responses at the $BMDL_{01}$ for each data set modeled appear not to be elevated over the control, the possibility of a small increase in response at these dose levels cannot be eliminated. Because the BMD approach is generally preferred to the NOAEL/LOAEL approach, and because the $BMDL_{01}$ values are similar to the NOAELs (difference of approximately 2-fold), the $BMDL_{01}$ values were used to estimate the PODs for offspring loss.

2.1.2.4.2 Pup Body Weight

See the Risk Evaluation document ([EPA-HQ-OPPT-2016-0735](#)) for details on this endpoint.

3 DOSE-RESPONSE MODELING FOR THE DERIVATION OF POINTS OF DEPARTURE

This appendix provides technical detail on dose-response evaluation and determination of points of departure (PODs) for relevant toxicological endpoints. The endpoints were modeled using the U.S. Environmental Protection Agency (EPA) Benchmark Dose Software (BMDS, version 2.6). This appendix describes the common practices used in evaluating the model fit and selecting the

appropriate model for determining the POD, as outlined in the Benchmark Dose Technical Guidance Document (U.S., 2012). In some cases, it may be appropriate to use alternative methods, based on statistical judgment; exceptions are noted as necessary in the summary of the modeling results.

3.1 Noncancer Endpoints for BMD Modeling

The noncancer endpoints that were selected for dose-response modeling are presented in Table 3-1. Noncancer endpoints selected for dose-response modeling for HBCD. For each endpoint, the doses and response data used for the modeling are presented.

Table 3-1. Noncancer endpoints selected for dose-response modeling for HBCD

Endpoint	Species (strain)/sex	Dose (mg/kg-d) ^a	Incidence [%] or mean \pm SD (number of animals or litters)	BMR(s)
Thyroid				
↓T4 Ema et al. (2008)	F0 rats (CRL Sprague-Dawley)/male	0 10 101 1,008 TWA of lifetime exposure, F0	4.04 \pm 1.42 (8) 3.98 \pm 0.89 (8) 2.97 \pm 0.76 (8) 2.49 \pm 0.55 (8)	10% RD, 15% RD, 20% RD, 1 SD
↓T4 Ema et al. (2008)	F0 rats (CRL Sprague-Dawley)/female	0 14 141 1,363 TWA of lifetime exposure, F0	2.84 \pm 0.61 (8) 3.14 \pm 0.48 (8) 3.00 \pm 0.77 (8) 1.96 \pm 0.55 (8)	10% RD, 15% RD, 20% RD, 1 SD
↓T4 Ema et al. (2008)	F1 rats (CRL Sprague-Dawley)/female	0 14.3 138 1,363 TWA of lifetime exposure, F1	3.59 \pm 1.08 (8) 3.56 \pm 0.53 (8) 3.39 \pm 1.21 (8) 2.58 \pm 0.37 (8)	10% RD, 15% RD, 20% RD, 1 SD
Liver				
Relative liver weight Ema et al. (2008)	F1 rats (CRL Sprague-Dawley)/male weanlings, PND 26	0 16.5 168 1,570 TWA of F0 gestational and lactational doses	4.6 \pm 0.37 (23) 4.6 \pm 0.32 (21) 5.05 \pm 0.32 (20) 6 \pm 0.44 (17)	10% RD, 1 SD
Relative liver weight Ema et al. (2008)	F1 rats (CRL Sprague-Dawley)/female weanlings, PND 26	0 16.5 168 1,570	4.57 \pm 0.35 (23) 4.59 \pm 0.28 (21) 5.02 \pm 0.32 (20) 6.07 \pm 0.36 (14)	10% RD, 1 SD

Endpoint	Species (strain)/sex	Dose (mg/kg-d) ^a	Incidence [%] or mean ± SD (number of animals or litters)	BMR(s)
		TWA of F0 gestational and lactational doses		
Relative liver weight Ema et al. (2008)	F1 rats (CRL Sprague-Dawley)/male adults	0 11.4 115 1,142 TWA of lifetime exposure, F1	3.27 ± 0.18 (24) 3.34 ± 0.26 (24) 3.37 ± 0.25 (22) 3.86 ± 0.28 (24)	10% RD, 1 SD
Relative liver weight Ema et al. (2008)	F1 rats (CRL Sprague-Dawley)/female adults	0 14.3 138 1,363 TWA of lifetime exposure, F1	4.18 ± 0.42 (22) 4.39 ± 0.44 (22) 4.38 ± 0.47 (20) 5.05 ± 0.50 (13)	10% RD, 1 SD
Relative liver weight Ema et al. (2008)	F2 rats (CRL Sprague-Dawley)/male weanlings, PND 26	0 14.7 139 1,360 TWA of F1 gestational and lactational doses	4.72 ± 0.59 (22) 4.74 ± 0.35 (22) 5.04 ± 0.4 (18) 6.0 ± 0.25 (13)	10% RD, 1 SD
Relative liver weight Ema et al. (2008)	F2 rats (CRL Sprague-Dawley)/female weanlings, PND 26	0 14.7 139 1,360 TWA of F1 gestational and lactational doses	4.70 ± 0.27 (21) 4.70 ± 0.28 (22) 4.94 ± 0.32 (20) 5.89 ± 0.44 (13)	10% RD, 1 SD
Relative liver weight and hepatocellular vacuolization WIL Research (2001)	Rats (Sprague-Dawley)/male	0 100 300 1,000	2.709 ± 0.1193 (10) 3.175 ± 0.2293 (10) 3.183 ± 0.2653 (10) 3.855 ± 0.1557 (9)	10% RD, 1 SD
Relative liver weight and hepatocellular vacuolization WIL Research (2001)	Rats (Sprague-Dawley)/female	0 100 300 1,000	2.887 ± 0.2062 (10) 3.583 ± 0.2734 (10) 3.578 ± 0.3454 (10) 4.314 ± 0.2869 (10)	10% RD, 1 SD
Reproductive				
Primordial follicles Ema et al. (2008) (supplemental)	F1 parental rat (CRL Sprague-Dawley)/female	0 9.6 96 941	316.3 ± 119.5 (10) 294.2 ± 66.3 (10) 197.9 ± 76.9 (10) 203.4 ± 79.5 (10)	1% ER, 5% ER, 10% ER

Endpoint	Species (strain)/sex	Dose (mg/kg-d) ^a	Incidence [%] or mean ± SD (number of animals or litters)	BMR(s)
		The F0 adult female gestational doses		
Incidence of non-pregnancy Ema et al. (2008)	F0 and F1 parental rats combined (CRL Sprague-Dawley)/female	0 13.3 132 1,302 TWA F0, F1 female pre-mating doses	1/48 [2%] 3/48 [6.2%] 7/48 [14.5%] 7/47 [14.9%]	5% ER, 10% ER
Developmental				
Offspring loss at PND 4 Ema et al. (2008)	F2 offspring rats (CRL Sprague-Dawley)	0 9.7 100 995 The F1 adult female gestational doses	28/132 [21%] 26/135 [19.3%] 23/118 [19.5%] 47/120 [39.2%]	1% ER, 5% ER
Offspring loss at PND 21 Ema et al. (2008)	F2 offspring rats (CRL Sprague-Dawley)	0 19.6 179 1,724 The F1 adult female lactational doses	11/70 [15.7%] 7/70 [10.0%] 18/64 [28.1%] 32/64 [50.0%]	1% ER, 5% ER
Pup weight during lactation at PND 21 Ema et al. (2008)	F2 offspring rats (CRL Sprague-Dawley)/male	0 19.6 179 1,724 The F1 adult female lactational doses	53 ± 12.6 (22) 56.2 ± 6.7 (22) 54.1 ± 10.1 (18) 42.6 ± 8.3 (13)	5% RD, 10% RD, 0.5 SD, 1 SD
Pup weight during lactation at PND 21 Ema et al. (2008)	F2 offspring rats (CRL Sprague-Dawley)/female	0 19.6 179 1,724 The F1 adult female lactational doses	52 ± 10 (21) 52.8 ± 6.6 (22) 51.2 ± 10.8 (20) 41.6 ± 8.4 (13)	5% RD, 10% RD, 0.5 SD, 1 SD

^aDoses were calculated as TWA doses using weekly average doses (in mg/kg-day) as reported in Table 10 of the Supplemental Materials to [Ema et al. \(2008\)](#).

BMR = benchmark response; ER = extra risk; PND = postnatal day; RD = relative deviation; SD = standard deviation; T4 = thyroxine; TWA = time-weighted average

3.2 Dose-Response Modeling of Non-Cancer Endpoints

3.2.1 Evaluation of Model Fit

For each dichotomous endpoint where only summary data (i.e., number affected and total number exposed per group) were available, BMDS dichotomous models¹ were fitted to the data using the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test (χ^2 p-value < 0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the benchmark response (BMR).

For each dichotomous endpoint for which incidence data were available for individual animals, BMDS nested dichotomous models² were fitted to the data using the maximum likelihood method. Each nested model was tested for goodness-of-fit using a bootstrap approach. Chi-square statistics were computed with both bootstrap iterations and original data. The p-value was the proportion of chi-square values from the iterations that were greater than the original chi-square value (χ^2 p-value < 0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

For each continuous endpoint, BMDS continuous models³ were fitted to the data using the maximum likelihood method. Model fit was assessed by a series of tests as follows. For each model, first the homogeneity of the variances was tested using a likelihood ratio test (BMDS Test 2). If Test 2 was not rejected (χ^2 p-value \geq 0.10), the model was fitted to the data assuming constant variance. If Test 2 was rejected (χ^2 p-value < 0.10), the variance was modeled as a power function of the mean, and the variance model was tested for adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using either constant variance or modeled variance, models for the mean response were tested for adequacy of fit using a likelihood ratio test (BMDS Test 4, with χ^2 p-value < 0.10 indicating inadequate fit). Other factors were also used to assess the model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

3.2.2 Model Selection

To select the appropriate model from which to derive the POD for each endpoint, the BMDL estimate (95% lower confidence limit on the benchmark dose [BMD], as estimated by the profile likelihood method) and Akaike's information criterion (AIC) value were used to select the model from among the models exhibiting adequate fit. If the BMDL estimates were "sufficiently close," that is, differed by at most 3-fold, the model selected was the one that yielded the lowest AIC

¹Unless otherwise specified, all available BMDS dichotomous models besides the alternative and nested dichotomous models were fitted. The following parameter restrictions were applied: for the LogLogistic model, restrict slope \geq 1; for the Gamma and Weibull models, restrict power \geq 1.

²Unless otherwise specified, all available BMDS nested dichotomous models were fitted. For the nested Logistic, NCTR, and Rai and van Ryzin models, power \geq 1 was applied.

³Unless otherwise specified, all available BMDS continuous models were fitted. The following parameter restrictions were applied: for the polynomial models, restrict the coefficients b1 and higher to be nonnegative or nonpositive if the direction of the adverse effect is upward or downward, respectively; for the Hill, Power, and Exponential models, restrict power \geq 1.

value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

For nested dichotomous models, there are the options of including a litter-specific covariate and estimating intralitter correlations, yielding four combinations of option selections, as displayed in Table 3-2. All the three nested dichotomous models were fitted for every combination in the table, yielding four sets of models (12 model runs in total).

Table 3-2. The combinations of option selections for the nested dichotomous models

Litter-specific covariates used Intralitter correlations estimated	Litter-specific covariates used Intralitter correlations assumed zero
Litter-specific covariates not used Intralitter correlations estimated	Litter-specific covariates not used Intralitter correlations assumed zero

The appropriate model was selected from this set of 12 models using the same procedure as for the non-nested models as described in Section 2.3.9 (page 39) of the Benchmark Dose Technical Guidance Document ([U.S., 2012](#)). If multiple litter specific covariates were tested, this same set of 12 modeling options was evaluated for each litter-specific covariate (e.g., litter size, implantation site, dam body weight) and the appropriate model was selected from the expanded set of modeling options ($12 \times$ number of litter-specific covariates considered) using the same procedure as for the non-nested models.

3.2.3 Modeling Results

Below are tables summarizing the modeling results for the noncancer endpoints modeled.

3.2.3.1 Thyroid

Table 3-3. Summary of BMD modeling results for T4 in F0 parental male CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008); BMR = 15% RD from control mean, 20% RD from control mean, and 1 SD change from control mean

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	BMD _{15RD} (mg/kg-d)	BMDL _{15RD} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3) ^b	0.0473	33.926	259	177	399	274	Of the models without saturation that provided an adequate fit and a valid BMDL estimate, the Exponential 4 model with modeled variance was selected based on lowest AIC (BMDLs differed by <3).
Exponential (M4) Exponential (M5)^c	0.742	29.933	23.9	6.99	39.1	11.5	
Hill	0.949	29.829	14.4	3.21	25.6	5.66	
Power ^d Polynomial 3 ^o ^e Polynomial 2 ^o ^f Linear	0.0418	34.174	303	227	455	341	
Model ^a	Goodness of fit		BMD _{20RD} (mg/kg-d)	BMDL _{20RD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)	
	p-value	AIC					
Exponential (M2) Exponential (M3) ^b	0.0473	33.926	548	376	866	511	
Exponential (M4) Exponential (M5) ^c	0.742	29.933	57.9	17.2	101	29.5	
Hill	0.949	29.829	42.0	9.11	94.9	Error ^g	
Power ^d Polynomial 3 ^o ^e Polynomial 2 ^o ^f Linear	0.0418	34.174	607	454	906	595	

^aModeled variance case presented (BMDS Test 2 p-value = 0.0756, BMDS Test 3 p-value = 0.553), selected model in bold; scaled residuals for selected model for doses 0, 10.2, 101, and 1,008 mg/kg-day were -0.1665, 0.166, 0.03642, and -0.03619, respectively.

^bFor the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

^cFor the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.

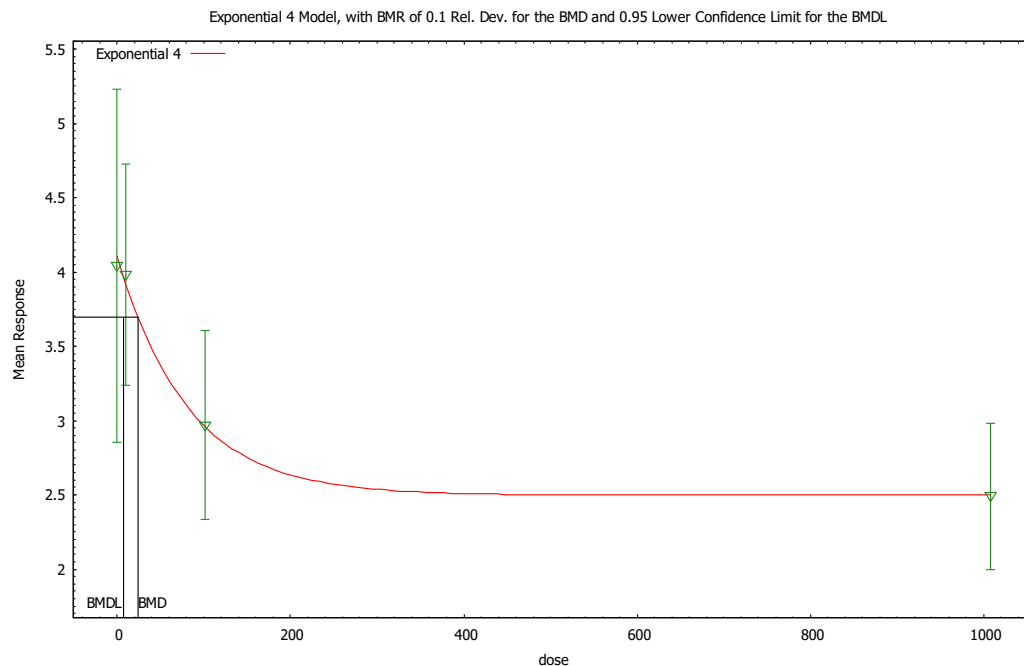
^dFor the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

^eFor the Polynomial 3^o model, the b3 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2^o model. For the Polynomial 3^o model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

^fFor the Polynomial 2^o model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

^gBMD or BMDL computation failed for this model.

Data from [Ema et al. \(2008\)](#)



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 BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure 3-1. Plot of mean response by dose, with fitted curve for Exponential 4 Model, for T4 in F0 parental CRL Sprague-Dawley male rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential 4 Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is:

$$\text{Model 4: } Y[\text{dose}] = a * [c - (c - 1) * \exp\{-b * \text{dose}\}]$$

A modeled variance is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 23.8946

BMDL at the 95% confidence level = 6.99406

Parameter Estimates

Variable	Estimate	Default initial parameter values
lalpha	-3.94284	-3.54227
rho	2.98463	2.72754
a	4.1075	4.242
b	0.0123219	0.00282274
d	1 (specified)	1 (specified)

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
------	---	---------------	----------------	-------------	--------------	------------------

0	8	4.04	4.11	1.42	1.15	-0.167
10.2	8	3.98	3.92	0.89	1.07	0.166
101	8	2.97	2.961	0.76	0.71	0.036
1,008	8	2.49	2.50	0.59	0.56	-0.036

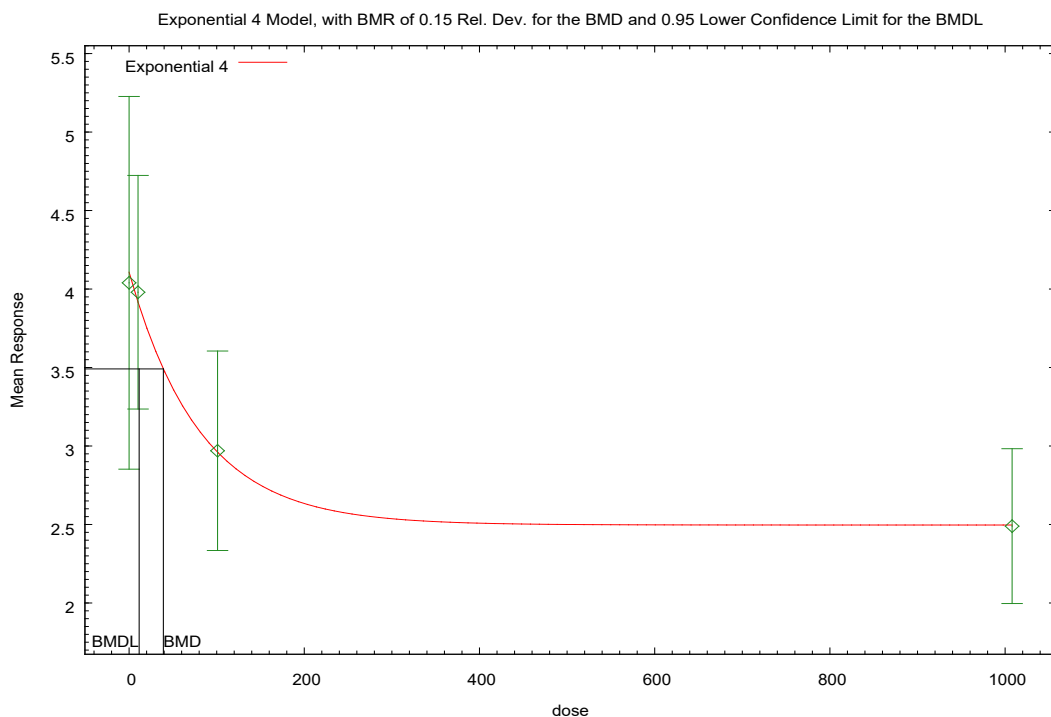
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-12.76333	5	35.52665
A2	-9.319925	8	34.63985
A3	-9.91228	6	31.82456
fitted	-9.966286	5	29.93257
R	-19.64317	2	43.28634

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	20.65	6	0.002123
Test 2	6.887	3	0.07559
Test 3	1.185	2	0.553
Test 6a	0.108	1	0.7424

df = degree(s) of freedom



11:24 08/18 2017

BMR = 15% RD from control mean; dose shown in mg/kg-day.

Figure 3-2. Plot of mean response by dose, with fitted curve for Exponential 4 Model, for T4 in F0 parental CRL Sprague-Dawley male rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential 4 Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is:

$$\text{Model 4: } Y[\text{dose}] = a * [c - (c - 1) * \exp\{-b * \text{dose}\}]$$

A modeled variance is fit

Benchmark Dose Computation

BMR = 15% RD

BMD = 39.1317

BMDL at the 95% confidence level = 11.5235

Parameter Estimates

Variable	Estimate	Default initial parameter values
lalpha	-3.94284	-3.54227
rho	2.98463	2.72754
a	4.1075	4.242
b	0.0123219	0.00282274
c	0.607906	0.55903

d	1 (specified)	1 (specified)
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Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	4.04	4.11	1.42	1.15	-0.167
10.2	8	3.98	3.92	0.89	1.07	0.166
101	8	2.97	2.961	0.76	0.71	0.036
1,008	8	2.49	2.50	0.59	0.55	-0.036

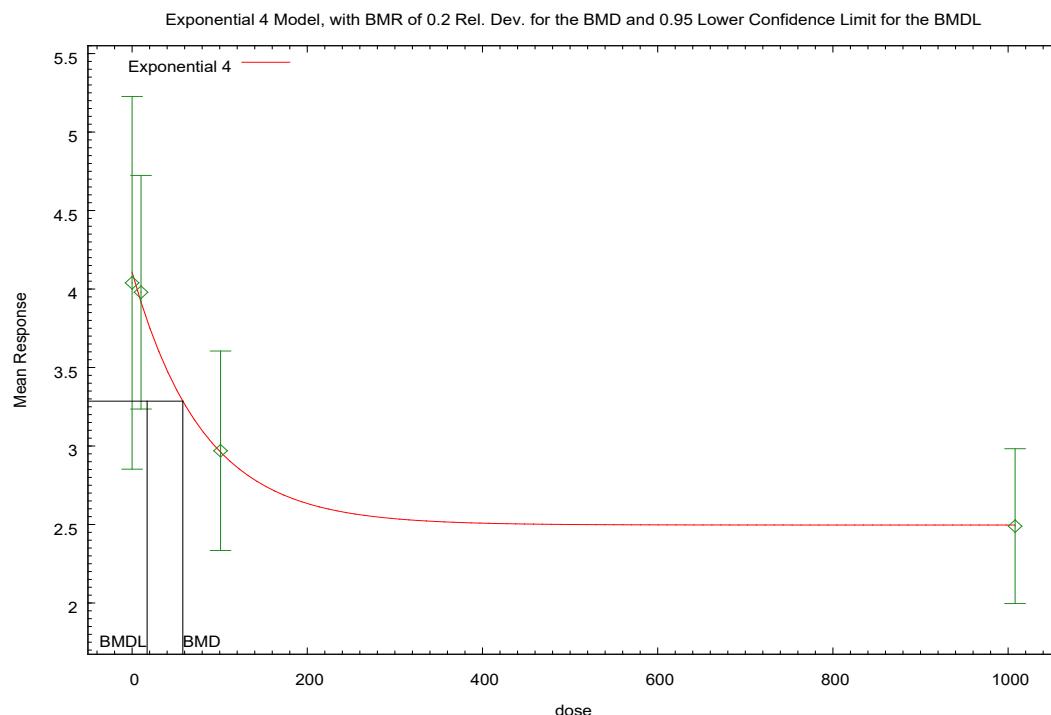
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-12.76333	5	35.52665
A2	-9.319925	8	34.63985
A3	-9.91228	6	31.82456
fitted	-9.966286	5	29.93257
R	-19.64317	2	43.28634

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	20.65	6	0.002123
Test 2	6.887	3	0.07559
Test 3	1.185	2	0.553
Test 6a	0.108	1	0.7424

df = degree(s) of freedom



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BMR = 20% RD from control mean; dose shown in mg/kg-day.

Figure 3-3. Plot of mean response by dose, with fitted curve for Exponential 4 Model, for T4 in F0 parental CRL Sprague-Dawley male rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential 4 Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is:

$$\text{Model 4: } Y[\text{dose}] = a * [c - (c - 1) * \exp\{-b * \text{dose}\}]$$

A modeled variance is fit

Benchmark Dose Computation

BMR = 20% RD

BMD = 57.9065

BMDL at the 95% confidence level = 17.1892

Parameter Estimates

Variable	Estimate	Default initial parameter values
lalpha	-3.94284	-3.54227
rho	2.98463	2.72754
a	4.1075	4.242
b	0.0123219	0.00282274
c	0.607906	0.55903
d	1 (specified)	1 (specified)

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	4.04	4.11	1.42	1.15	-0.167
10.2	8	3.98	3.92	0.89	1.07	0.166
101	8	2.97	2.961	0.76	0.71	0.036
1,008	8	2.49	2.50	0.59	0.55	-0.036

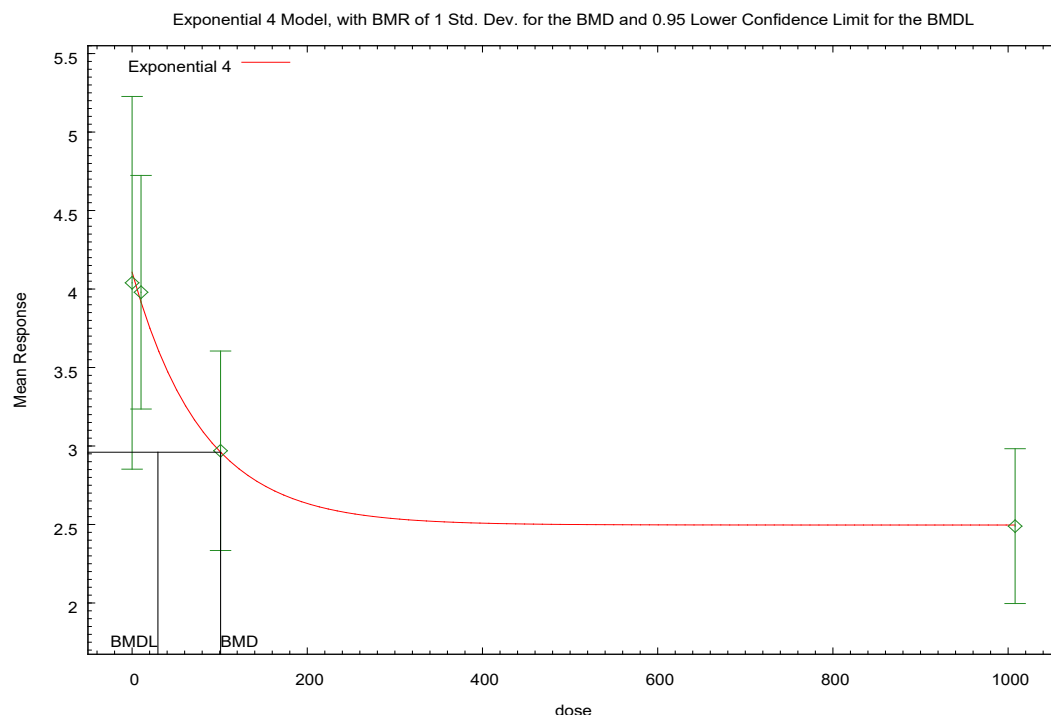
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-12.76333	5	35.52665
A2	-9.319925	8	34.63985
A3	-9.91228	6	31.82456
fitted	-9.966286	5	29.93257
R	-19.64317	2	43.28634

Tests of Interest

Test	$-2*\log$ (likelihood ratio)	Test df	p-value
Test 1	20.65	6	0.002123
Test 2	6.887	3	0.07559
Test 3	1.185	2	0.553
Test 6a	0.108	1	0.7424

df = degree(s) of freedom



11:24 08/18 2017

BMR = 1 SD from control mean; dose shown in mg/kg-day.

Figure 3-4. Plot of mean response by dose, with fitted curve for Exponential 4 Model, for T4 in F0 parental CRL Sprague-Dawley male rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential 4 Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is:

$$\text{Model 4: } Y[\text{dose}] = a * [c - (c - 1) * \exp\{-b * \text{dose}\}]$$

A modeled variance is fit

Benchmark Dose Computation

BMR = 1 SD

BMD = 101.035

BMDL at the 95% confidence level = 29.4693

Parameter Estimates

Variable	Estimate	Default initial parameter values
lalpha	-3.94284	-3.54227
rho	2.98463	2.72754
a	4.1075	4.242
b	0.0123219	0.00282274
c	0.607906	0.55903
d	1 (specified)	1 (specified)

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	4.04	4.11	1.42	1.15	-0.167
10.2	8	3.98	3.92	0.89	1.07	0.166
101	8	2.97	2.961	0.76	0.71	0.036
1,008	8	2.49	2.50	0.59	0.55	-0.036

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-12.76333	5	35.52665
A2	-9.319925	8	34.63985
A3	-9.91228	6	31.82456
fitted	-9.966286	5	29.93257
R	-19.64317	2	43.28634

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	20.65	6	0.002123
Test 2	6.887	3	0.07559
Test 3	1.185	2	0.553
Test 6a	0.108	1	0.7424

df = degree(s) of freedom

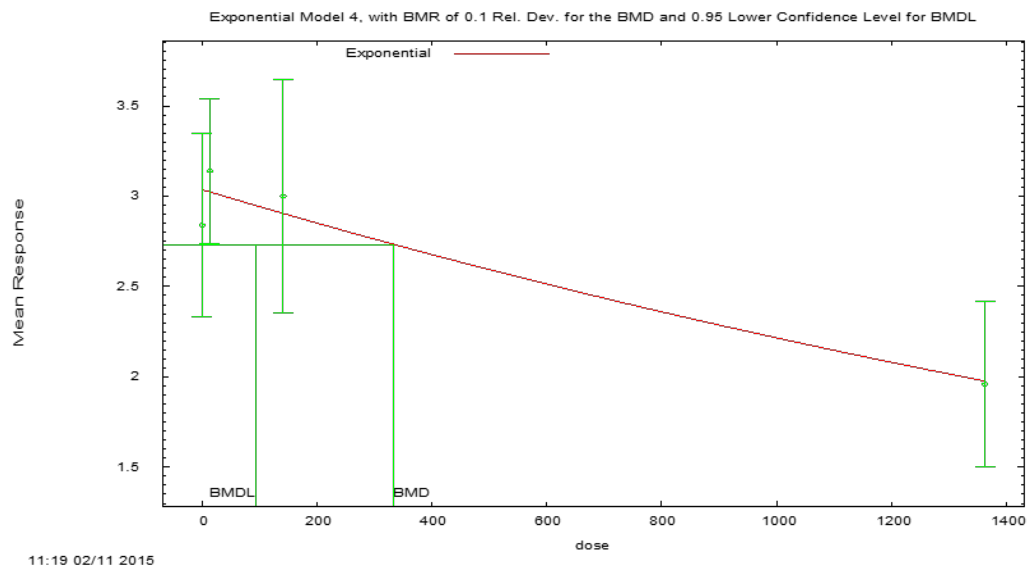
Table 3-4. Summary of BMD modeling results for T4 in F0 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008); BMR = 10% RD from control mean, 15% RD from control mean, 20% RD from control mean, and 1 SD change from control mean

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	BMD _{15RD} (mg/kg-d)	BMDL _{15RD} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2)	0.479	3.7677	334	225	516	348	
Exponential (M3)	0.298	5.3774	1,065	232	1,150	357	
Exponential (M4)	0.479	3.7677	334	93.8	516	154	
Exponential (M5)	N/A ^b	7.3774	1,086	103	1,158	143	
Hill	N/A ^b	7.3774	1,067	100	1,138	error ^c	
Power	0.298	5.3774	1,171	293	1,230	439	
Polynomial 3 ^o	0.582	3.3778	902	816	1,032	934	
Polynomial 2 ^o	0.580	3.3836	733	293	897	439	
Linear	0.505	3.6625	389	289	584	433	
Model ^a	Goodness of fit		BMD _{20RD} (mg/kg-d)	BMDL _{20RD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)	
	p-value	AIC					
Exponential (M2)	0.479	3.7677	708	477	680	433	
Exponential (M3)	0.298	5.3774	1,240	491	1,234	446	
Exponential (M4)	0.479	3.7677	708	229	680	211	
Exponential (M5)	N/A ^b	7.3774	1,217	146	1,211	145	
Hill	N/A ^b	7.3774	1,185	error ^c	1,178	error ^c	
Power	0.298	5.3774	1,275	586	1,270	532	
Polynomial 3 ^o	0.582	3.3778	1,136	1,028	1,126	999	
Polynomial 2 ^o	0.580	3.3836	1,036	586	1,021	532	
Linear	0.505	3.6625	779	577	751	523	

^aConstant variance case presented (BMDS Test 2 p-value = 0.579), selected model in bold; scaled residuals for selected model for doses 0, 14, 141.3, and 1,363 mg/kg-day were -0.9501, 0.5631, 0.4611, and -0.07911, respectively.

^bNo available degrees of freedom to calculate a goodness-of-fit value.

^cBMD or BMDL computation failed for this model.



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure 3-5. Plot of mean response by dose, with fitted curve for Exponential Model 4, for T4 in F0 parental CRL Sprague-Dawley female rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 334.313

BMDL at the 95% confidence level = 93.781

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-1.06976	-1.11576
rho(S)	N/A	0
a	3.03677	3.297
b	0.000315155	0.00199958
c	0	0.566171
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	2.84	3.037	0.61	0.5857	-0.9501

14	8	3.14	3.023	0.48	0.5857	0.5631
141.3	8	3	2.905	0.77	0.5857	0.4611
1,363	8	1.96	1.976	0.55	0.5857	-0.07911

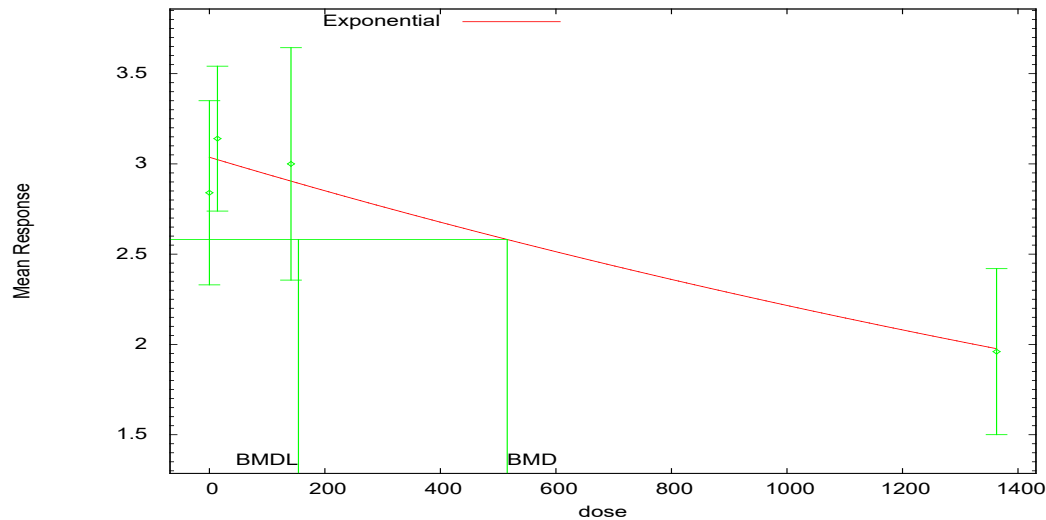
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	1.852186	5	6.295628
A2	2.83624	8	10.32752
A3	1.852186	5	6.295628
R	-6.115539	2	16.23108
4	1.116152	3	3.767695

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	17.9	6	0.006478
Test 2	1.968	3	0.5791
Test 3	1.968	3	0.5791
Test 6a	1.472	2	0.479

Exponential Model 4, with BMR of 0.15 Rel. Dev. for the BMD and 0.95 Lower Confidence Level for BA



11:21 02/11 2015
 BMR = 15% RD from control mean; dose shown in mg/kg-day.

Figure 3-6. Plot of mean response by dose, with fitted curve for Exponential Model 4, for T4 in F0 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 15% RD

BMD = 515.679

BMDL at the 95% confidence level = 154.19

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-1.06976	-1.11576
rho(S)	N/A	0
a	3.03677	3.297
b	0.000315155	0.00199958
c	0	0.566171
d	1	1

Table of Data and Estimated Values of Interest

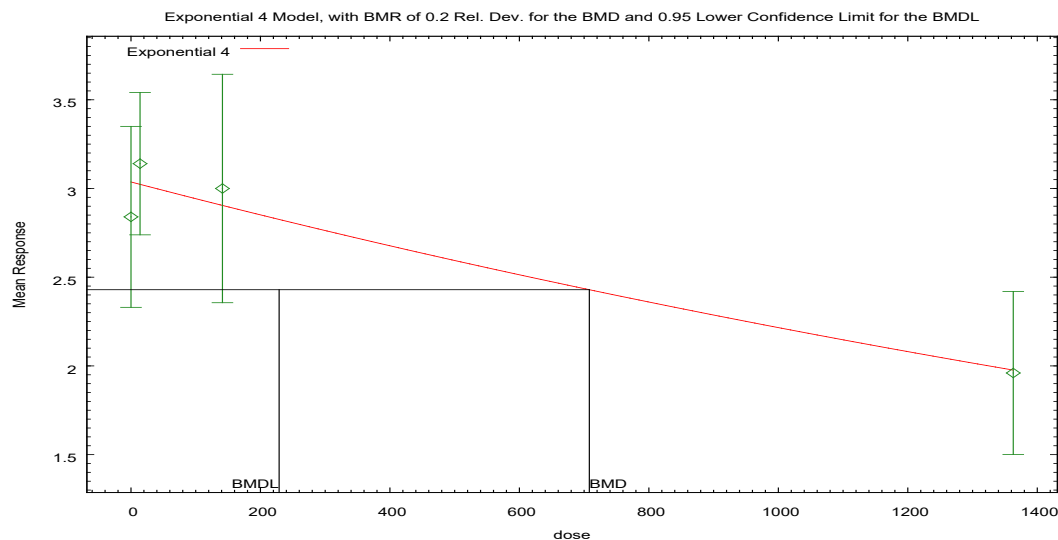
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	2.84	3.037	0.61	0.5857	-0.9501
14	8	3.14	3.023	0.48	0.5857	0.5631
141.3	8	3	2.905	0.77	0.5857	0.4611
1,363	8	1.96	1.976	0.55	0.5857	-0.07911

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	1.852186	5	6.295628
A2	2.83624	8	10.32752
A3	1.852186	5	6.295628
R	-6.115539	2	16.23108
4	1.116152	3	3.767695

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	17.9	6	0.006478
Test 2	1.968	3	0.5791
Test 3	1.968	3	0.5791
Test 6a	1.472	2	0.479



BMR = 20% RD from control mean; dose shown in mg/kg-day.

Figure 3-7. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for T4 in F0 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 20% RD

BMD = 708.043

BMDL at the 95% confidence level = 228.829

Parameter Estimates

Variable	Estimate	Default initial parameter values
Lnalpha	-1.06976	-1.11576
Rho	N/A	0
A	3.03677	3.297
B	0.000315155	0.00199958
C	0	0.566171
D	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	2.84	3.04	0.61	0.59	-0.9501
14	8	3.14	3.02	0.48	0.59	0.5631

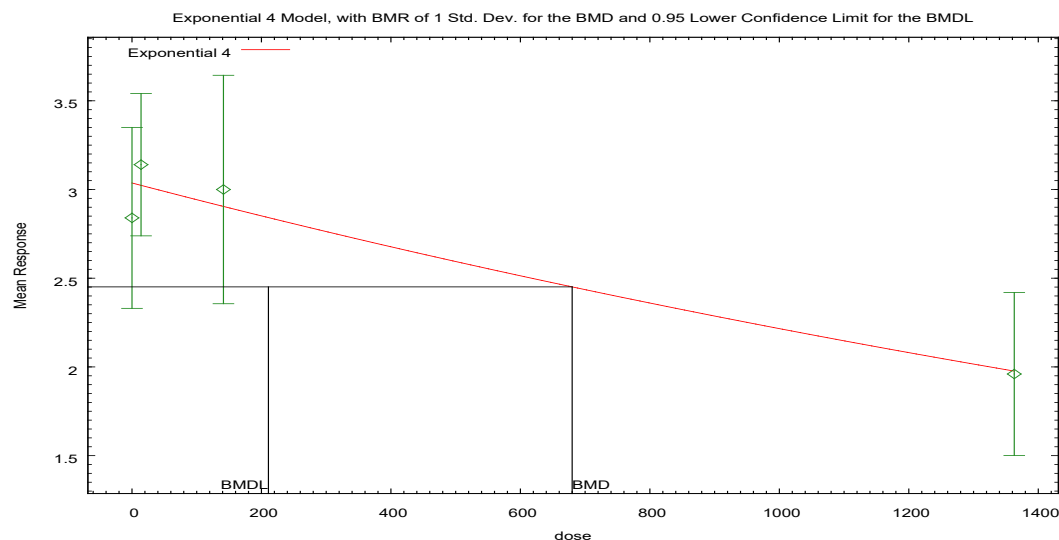
141.3	8	3	2.9	0.77	0.59	0.4611
1,363	8	1.96	1.98	0.55	0.59	-0.07911

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	1.852186	5	6.295628
A2	2.83624	8	10.32752
A3	1.852186	5	6.295628
R	-6.115539	2	16.23108
4	1.116152	3	3.767695

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	17.9	6	0.006478
Test 2	1.968	3	0.5791
Test 3	1.968	3	0.5791
Test 6a	1.472	2	0.479



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure 3-8. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for T4 in F0 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 679.939

BMDL at the 95% confidence level = 210.769

Parameter Estimates

Variable	Estimate	Default initial parameter values
Lalpha	-1.06976	-1.11576
Rho	N/A	0
A	3.03677	3.297
B	0.000315155	0.00199958
C	0	0.566171
D	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	2.84	3.04	0.61	0.59	-0.9501
14	8	3.14	3.02	0.48	0.59	0.5631
141.3	8	3	2.9	0.77	0.59	0.4611
1,363	8	1.96	1.98	0.55	0.59	-0.07911

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	1.852186	5	6.295628
A2	2.83624	8	10.32752
A3	1.852186	5	6.295628
R	-6.115539	2	16.23108
4	1.116152	3	3.767695

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	17.9	6	0.006478
Test 2	1.968	3	0.5791
Test 3	1.968	3	0.5791
Test 6a	1.472	2	0.479

Table 3-5. Summary of BMD modeling results for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks ([Ema et al., 2008](#)); BMR = 10% RD

from control mean, 15% RD from control mean, 20% RD from control mean, and 1 SD change from control mean

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	BMD _{15RD} (mg/kg-d)	BMDL _{15RD} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2)	0.305	19.978	448	320	691	493	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 (modeled variance) model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M3)	0.191	21.318	1,184	333	1,254	514	
Exponential (M4)	0.305	19.978	448	127	691	214	
Exponential (M5)	N/A ^b	23.318	1,193	153	1,259	144	
Hill	N/A ^b	23.318	1,131	153	1,204	error ^c	
Power	0.191	21.318	1,287	389	1,318	583	
Polynomial 3 ^o	0.424	19.323	984	898	1,127	1,028	
Polynomial 2 ^o	0.414	19.368	835	728	1,023	892	
Linear	0.323	19.868	498	379	747	568	
Model ^a	Goodness of fit		BMD _{20RD} (mg/kg-d)	BMDL _{20RD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)	
	p-value	AIC					
Exponential (M2)	0.305	19.978	948	677	1,344	828	
Exponential (M3)	0.191	21.318	1,305	705	1,362	876	
Exponential (M4)	0.305	19.978	948	328	1,344	536	
Exponential (M5)	N/A ^b	23.318	1,309	148	1,362	152	
Hill	N/A ^b	23.318	1,269	error ^c	1,360	error ^c	
Power	0.191	21.318	1,341	777	1,363	932	
Polynomial 3 ^o	0.424	19.323	1,240	1,132	1,360	1,193	
Polynomial 2 ^o	0.414	19.368	1,181	1,030	1,357	1,115	
Linear	0.323	19.868	996	757	1,344	896	

^aModeled variance case presented (BMDS Test 2 p-value = 0.00445), selected model in bold; scaled residuals for selected model for doses 0, 14.3, 138.3, and 1,363 mg/kg-day were 0.105, 0.05257, -0.1637, and 0.008804, respectively.

^bNo available degrees of freedom to calculate a goodness-of-fit value.

^cBMD or BMDL computation failed for this model.

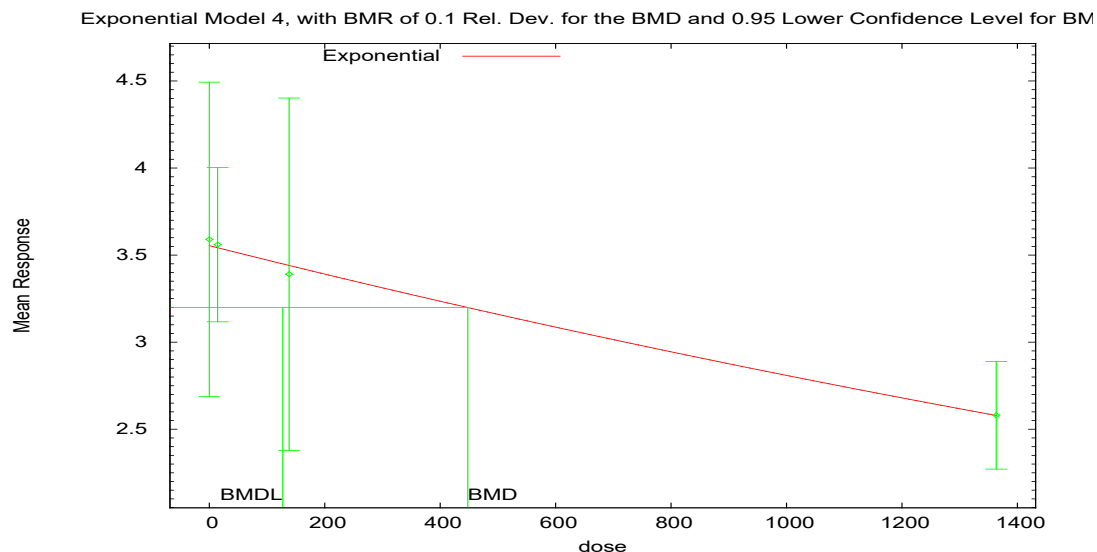


Figure 3-9. Plot of mean response by dose, with fitted curve for Exponential Model 4 (modeled variance) for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A modeled variance is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 447.782

BMDL at the 95% confidence level = 127.272

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-7.9144	-6.73265
rho	6.1823	5.13248
a	3.55422	3.7695
b	0.000235294	0.000283737
c	0	0.000684441
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	3.59	3.554	1.08	0.9635	0.105
14.3	8	3.56	3.542	0.53	0.9535	0.05257

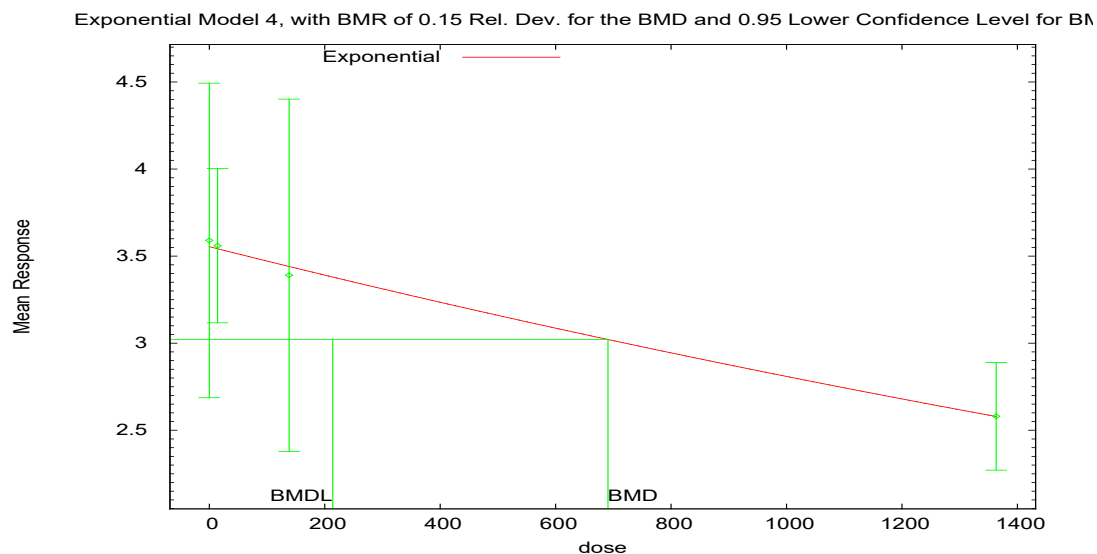
138.3	8	3.39	3.44	1.21	0.8713	-0.1637
1,363	8	2.58	2.579	0.37	0.3574	0.008804

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-9.516133	5	29.03227
A2	-2.971105	8	21.94221
A3	-4.802103	6	21.60421
R	-13.13332	2	30.26663
4	-5.988946	4	19.97789

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	20.32	6	0.002424
Test 2	13.09	3	0.004446
Test 3	3.662	2	0.1603
Test 6a	2.374	2	0.3052



BMR = 15% RD from control mean; dose shown in mg/kg-day.

Figure 3-10. Plot of mean response by dose, with fitted curve for Exponential Model 4, for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A modeled variance is fit

Benchmark Dose Computation

BMR = 15% RD

BMD = 690.705

BMDL at the 95% confidence level = 213.844

Parameter Estimates

Variable	Estimate	Default initial parameter values
Lalpha	-7.9144	-6.73265
Rho	6.1823	5.13248
A	3.55422	3.7695
B	0.000235294	0.000283737
C	0	0.000684441
D	1	1

Table of Data and Estimated Values of Interest

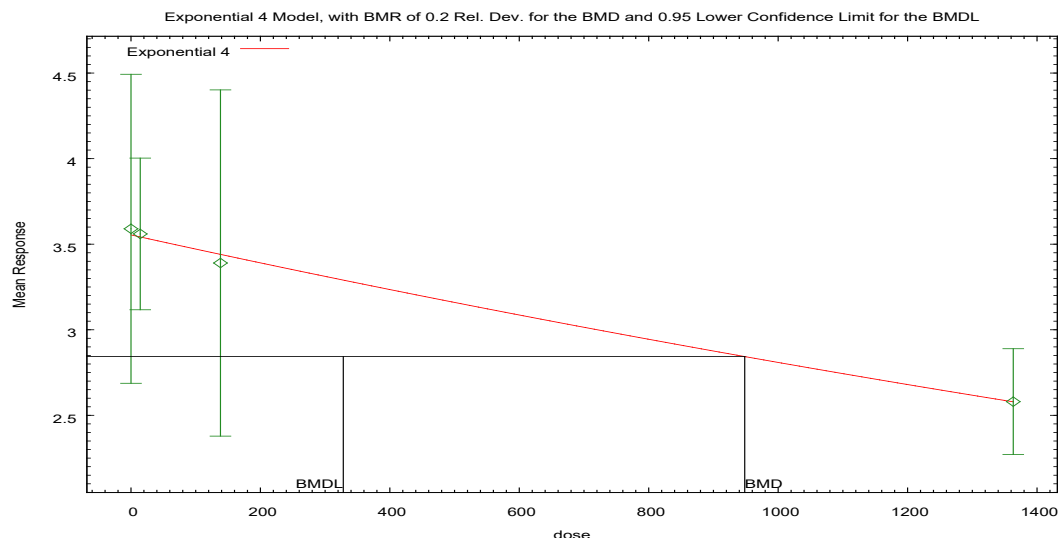
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	3.59	3.554	1.08	0.9635	0.105
14.3	8	3.56	3.542	0.53	0.9535	0.05257
138.3	8	3.39	3.44	1.21	0.8713	-0.1637
1,363	8	2.58	2.579	0.37	0.3574	0.008804

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-9.516133	5	29.03227
A2	-2.971105	8	21.94221
A3	-4.802103	6	21.60421
R	-13.13332	2	30.26663
4	-5.988946	4	19.97789

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	20.32	6	0.002424
Test 2	13.09	3	0.004446
Test 3	3.662	2	0.1603
Test 6a	2.374	2	0.3052



BMR = 20% RD from control mean; dose shown in mg/kg-day.

Figure 3-11. Plot of mean response by dose with fitted curve for Exponential (M4) model with modeled variance for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A modeled variance is fit

Benchmark Dose Computation

BMR = 20% RD

BMD = 948.359

BMDL at the 95% confidence level = 328.063

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-7.9144	-6.73265
rho	6.1823	5.13248
a	3.55422	3.7695
b	0.000235294	0.000283737
c	0	0.000684441
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	3.59	3.55	1.08	0.96	0.105
14.3	8	3.56	3.54	0.53	0.95	0.05257

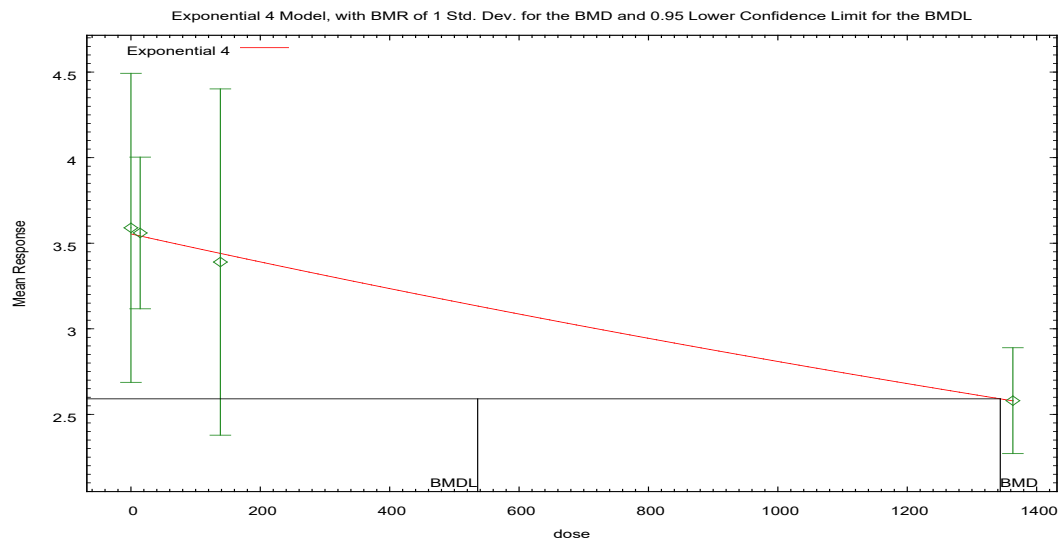
138.3	8	3.39	3.44	1.21	0.87	-0.1637
1,363	8	2.58	2.58	0.37	0.36	0.008804

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-9.516133	5	29.03227
A2	-2.971105	8	21.94221
A3	-4.802103	6	21.60421
R	-13.13332	2	30.26663
4	-5.988946	4	19.97789

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	20.32	6	0.002424
Test 2	13.09	3	0.004446
Test 3	3.662	2	0.1603
Test 6a	2.374	2	0.3052



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure 3-12. Plot of mean response by dose with fitted curve for Exponential (M4) model with modeled variance for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A modeled variance is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 1,343.81

BMDL at the 95% confidence level = 536.006

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-7.9144	-6.73265
rho	6.1823	5.13248
a	3.55422	3.7695
b	0.000235294	0.000283737
c	0	0.000684441
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	3.59	3.55	1.08	0.96	0.105
14.3	8	3.56	3.54	0.53	0.95	0.05257
138.3	8	3.39	3.44	1.21	0.87	-0.1637
1,363	8	2.58	2.58	0.37	0.36	0.008804

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-9.516133	5	29.03227
A2	-2.971105	8	21.94221
A3	-4.802103	6	21.60421
R	-13.13332	2	30.26663
4	-5.988946	4	19.97789

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	20.32	6	0.002424
Test 2	13.09	3	0.004446
Test 3	3.662	2	0.1603
Test 6a	2.374	2	0.3052

3.2.3.2 Liver

Table 3-6. Summary of BMD modeling results for relative liver weight (g/100 g BW) in male F1 CRL rats exposed to HBCD on GD 0–PND 26, dose TWA gestation through lactation (Ema et al., 2008); BMR = 10% RD from control mean and 1 SD change from control mean

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3) ^b	0.00369	-70.405	599	533	488	417	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest AIC and visual fit.
Exponential (M4)	0.606	-79.345	163	109	120	80.5	
Exponential (M5)	N/A ^c	-77.611	169	111	157	82.0	
Hill	N/A ^c	-77.611	169	104	156	75.4	
Powerd Polynomial 3 ^{oe} Polynomial 2 ^{of} Linear	0.00590	-71.344	548	480	440	371	

^aConstant variance case presented (BMDS Test 2 p-value = 0.462), selected model in bold; scaled residuals for selected model for doses 0, 16.5, 168, and 1,570 mg/kg-day were 0.3267, -0.3947, 0.05759, and -0.003788, respectively.

^bFor the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

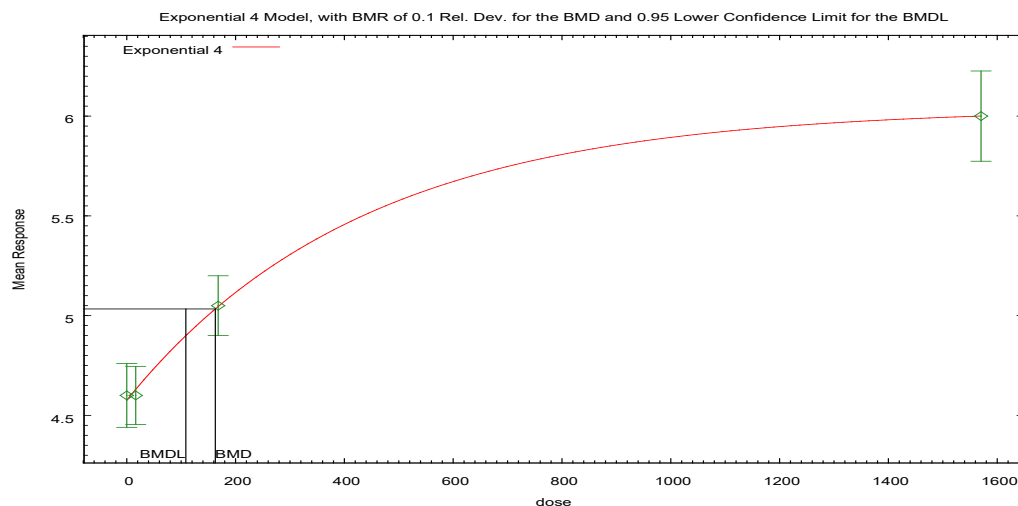
^cNo available degrees of freedom to calculate a goodness-of-fit value.

^dFor the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

^eFor the Polynomial 3^o model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

^fFor the Polynomial 2^o model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from [Ema et al. \(2008\)](#)



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure 3-13. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation through lactation (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 162.81

BMDL at the 95% confidence level = 108.569

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-2.07833	-2.08162
rho	N/A	0
a	4.5759	4.37
b	0.00230233	0.00120199
c	1.3199	1.44165
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	23	4.6	4.576	0.37	0.3538	0.3267
16.5	21	4.6	4.63	0.32	0.3538	-0.3947

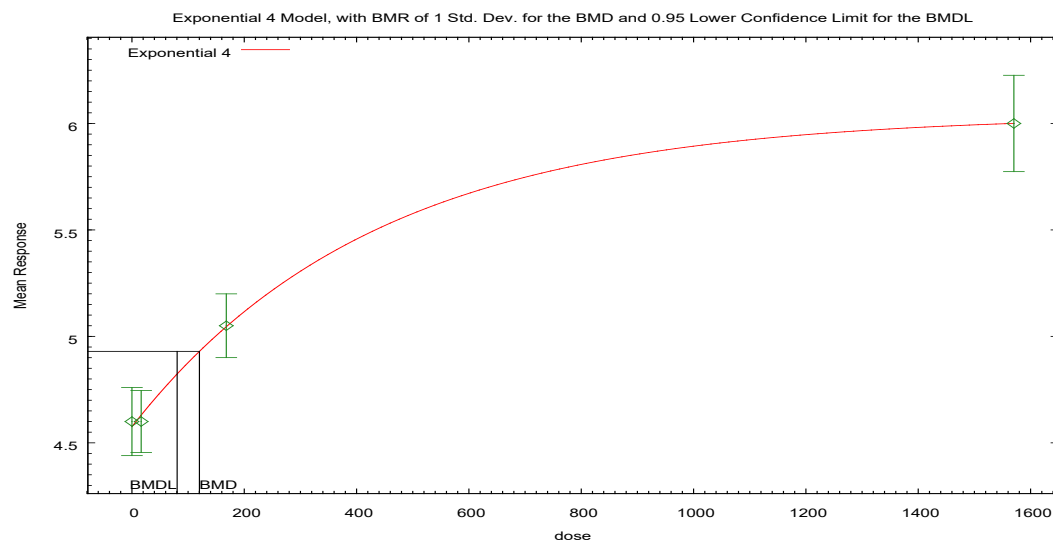
168	20	5.05	5.045	0.32	0.3538	0.05759
1,570	17	6	6	0.44	0.3538	-0.003788

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	43.80548	5	-77.61096
A2	45.09301	8	-74.18602
A3	43.80548	5	-77.61096
R	-5.569318	2	15.13864
4	43.67234	4	-79.34469

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	101.3	6	<0.0001
Test 2	2.575	3	0.4619
Test 3	2.575	3	0.4619
Test 6a	0.2663	1	0.6058



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure 3-14. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation through lactation (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 120.152

BMDL at the 95% confidence level = 80.5016

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-2.07833	-2.08162
rho	N/A	0
a	4.5759	4.37
b	0.00230233	0.00120199
c	1.3199	1.44165
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	23	4.6	4.576	0.37	0.3538	0.3267
16.5	21	4.6	4.63	0.32	0.3538	-0.3947
168	20	5.05	5.045	0.32	0.3538	0.05759
1,570	17	6	6	0.44	0.3538	-0.003788

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	43.80548	5	-77.61096
A2	45.09301	8	-74.18602
A3	43.80548	5	-77.61096
R	-5.569318	2	15.13864
4	43.67234	4	-79.34469

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	101.3	6	<0.0001
Test 2	2.575	3	0.4619
Test 3	2.575	3	0.4619
Test 6a	0.2663	1	0.6058

Table 3-7. Summary of BMD modeling results for relative liver weight (g/100 g BW) in F1 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose

TWA of gestation and lactation (Ema et al., 2008); BMR = 10% RD from control mean and 1 SD change from control mean

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3) ^b	0.00217	-82.410	560	503	418	359	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest AIC.
Exponential (M4)	0.731	-92.555	165	115	109	75.8	
Exponential (M5)	N/A ^c	-90.673	170	116	126	76.4	
Hill	N/A ^c	-90.673	170	110	124	70.8	
Power ^d Polynomial 3 ^o ^e Polynomial 2 ^o ^f Linear ^g	0.00403	-83.646	507	449	371	315	

^aConstant variance case presented (BMDs Test 2 p-value = 0.711), selected model in bold; scaled residuals for selected model for doses 0, 16.5, 168, and 1,570 mg/kg-day were 0.2185, -0.263, 0.03719, and -0.002332, respectively.

^bFor the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

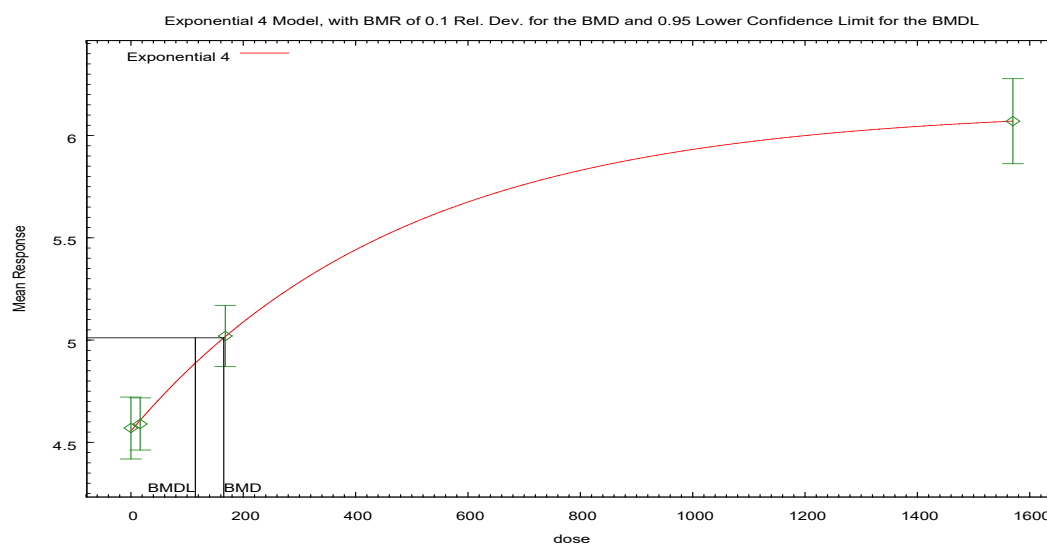
^cNo available degrees of freedom to calculate a goodness-of-fit value.

^dThe Power model may appear equivalent to the Linear model; however, differences exist in digits not displayed in the table.

^eFor the Polynomial 3^o model, the b3 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2^o model.

^fThe Polynomial 2^o model may appear equivalent to the Linear model; however, differences exist in digits not displayed in the table.

^gThe Linear model may appear equivalent to the Power model; however, differences exist in digits not displayed in the table. This also applies to the Polynomial 3^o and Polynomial 2^o models.



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BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure 3-15. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 weanling female CRL

Sprague-Dawley rats exposed to HBCD GD 0–PND 26, dose TWA of gestation and lactation (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 165.267

BMDL at the 95% confidence level = 114.71

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-2.28916	-2.29068
rho	N/A	0
a	4.5555	4.3415
b	0.00206359	0.00122548
c	1.34605	1.46804
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	23	4.57	4.555	0.35	0.3184	0.2185
16.5	21	4.59	4.608	0.28	0.3184	-0.263
168	20	5.02	5.017	0.32	0.3184	0.03719
1,570	14	6.07	6.07	0.36	0.3184	-0.002332

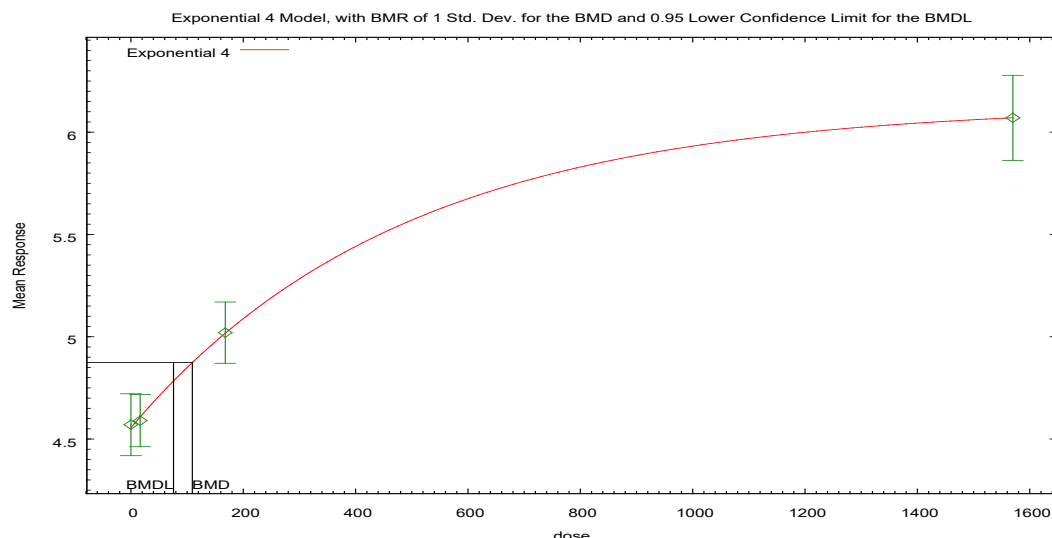
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	50.33659	5	-90.67319
A2	51.02517	8	-86.05034
A3	50.33659	5	-90.67319
R	-3.746671	2	11.49334
4	50.2774	4	-92.55481

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	109.5	6	<0.0001
Test 2	1.377	3	0.7109

Test 3	1.377	3	0.7109
Test 6a	0.1184	1	0.7308



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure 3-16. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA of gestation and lactation (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c-1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 109.314

BMDL at the 95% confidence level = 75.8445

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-2.28916	-2.29068
rho	N/A	0
a	4.5555	4.3415
b	0.00206359	0.00122548
c	1.34605	1.46804
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	23	4.57	4.555	0.35	0.3184	0.2185
16.5	21	4.59	4.608	0.28	0.3184	-0.263
168	20	5.02	5.017	0.32	0.3184	0.03719
1,570	14	6.07	6.07	0.36	0.3184	-0.002332

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	50.33659	5	-90.67319
A2	51.02517	8	-86.05034
A3	50.33659	5	-90.67319
R	-3.746671	2	11.49334
4	50.2774	4	-92.55481

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	109.5	6	<0.0001
Test 2	1.377	3	0.7109
Test 3	1.377	3	0.7109
Test 6a	0.1184	1	0.7308

Table 3-8. Summary of BMD modeling results for relative liver weight (g/100 g BW) in F1 adult male CRL Sprague-Dawley rats exposed to HBCD by diet for 15 weeks (Ema et al., 2008); BMR = 10% RD from control mean and 1 SD change from control mean.

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3) ^b	0.626	-167.34	703	601	519	433	Of the models that provided an adequate fit and a valid BMDL estimate, the Linear constant variance model was selected based on lowest AIC (BMDLs differed by <3). Exponential M5 and Hill models were excluded because both were saturated
Exponential (M4)	0.366	-165.46	578	243	402	161	
Exponential (M5)	0.366	-165.46	578	121	402	118	
Hill	0.367	-165.46	582	error ^c	404	164	
Power ^d Polynomial 3 ^{oe} Polynomial 2 ^{of} Linear	0.638	-167.38	680	573	496	409	

							models in this case.
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^aConstant variance case presented (BMDS Test 2 p-value = 0.181), selected model in bold; scaled residuals for selected model for doses 0, 11.4, 115, and 1,142 mg/kg-day were -0.723, 0.587, 0.165, and -0.0218, respectively.

^bFor the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

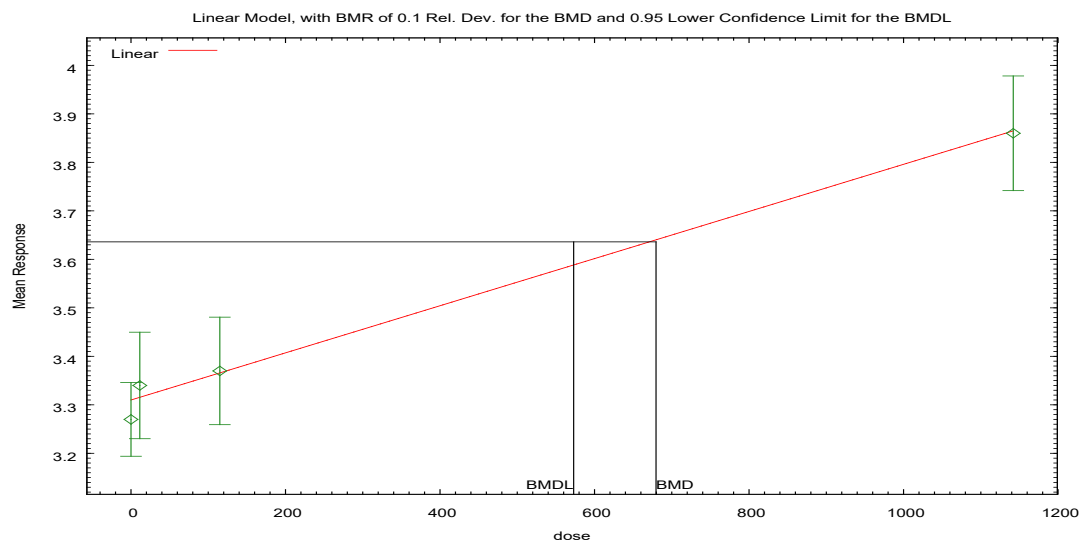
^cBMD or BMDL computation failed for this model.

^dFor the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

^eFor the Polynomial 3° model, the b3 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

^fFor the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from [Ema et al. \(2008\)](#)



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure 3-17. Plot of mean response by dose with fitted curve for Linear model with constant variance for relative liver weight (g/100 g BW) in F1 adult male CRL Sprague-Dawley rats exposed to HBCD by diet for 15 weeks ([Ema et al., 2008](#)).

Polynomial Model. (Version: 2.20; Date: 10/22/2014)

The form of the response function is: $Y[\text{dose}] = \text{beta}_0 + \text{beta}_1 * \text{dose}$

A constant variance model is fit

Benchmark Dose Computation.

BMR = 10% Relative deviation

BMD = 679.573

BMDL at the 95% confidence level = 572.977

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
alpha	0.0581671	0.0601744
rho	n/a	0
beta_0	3.30558	3.30581
beta_1	0.00048642	0.000486264

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	24	3.27	3.31	0.18	0.241	-0.723
11.4	24	3.34	3.31	0.26	0.241	0.587
115	22	3.37	3.36	0.25	0.241	0.165
1142	24	3.86	3.86	0.28	0.241	-0.0218

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	87.137654	5	-164.275308
A2	89.578448	8	-163.156897
A3	87.137654	5	-164.275308
fitted	86.688502	3	-167.377004
R	55.373159	2	-106.746318

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	68.4106	6	<0.0001
Test 2	4.88159	3	0.1807
Test 3	4.88159	3	0.1807
Test 4	0.898304	2	0.6382

Table 3-9. Summary of BMD modeling results for relative liver weight (g/100g bw) in F1 adult female CRL Sprague-Dawley rats exposed to HBCD by diet for 17 weeks (Ema et al., 2008); BMR = 10% RD from control mean and 1 SD change from control mean

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3) ^b	0.311	-40.783	791	615	824	635	Of the models that provided an adequate fit and a valid BMDL estimate, the
Exponential (M4) Exponential (M5) ^c	0.139	-38.934	569	184	603	203	

Hill	0.139	-38.937	575	186	610	208	Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3). Hill model was excluded because it was a saturated model in this case.
Power ^d	0.316	-40.816	761	578	795	598	
Polynomial 3 ^{oe}							
Polynomial 2 ^{of}							
Linear							

^aConstant variance case presented (BMD5 Test 2 p-value = 0.917), selected model in bold; scaled residuals for selected model for doses 0, 14.3, 138, and 1,363 mg/kg-d were -0.9658, 1.098, -0.1406, and 0.002993, respectively.

^bFor the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

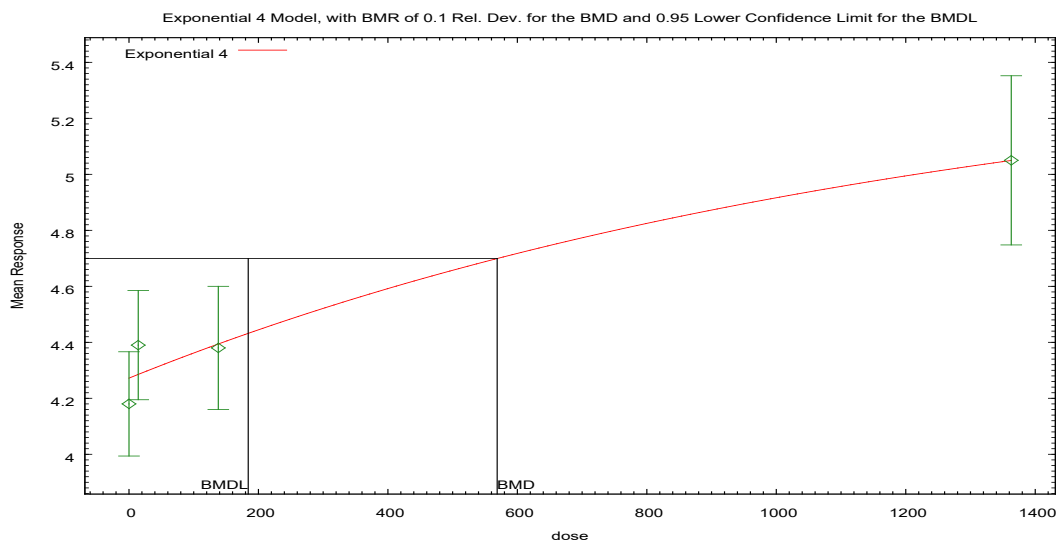
^cThe Exponential (M5) model may appear equivalent to the Exponential (M4) model; however, differences exist in digits not displayed in the table.

^dFor the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

^eFor the Polynomial 3^o model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

^fFor the Polynomial 2^o model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from [Ema et al. \(2008\)](#)



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BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure 3-18. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 adult female CRL Sprague-Dawley rats exposed to HBCD by diet for 17 weeks (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c-1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 568.784

BMDL at the 95% confidence level = 184.198

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-1.60953	-1.63795
rho	N/A	0
a	4.27208	3.971
b	0.000792725	0.0012372
c	1.27553	1.33531
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.18	4.272	0.42	0.4472	-0.9658
14.3	22	4.39	4.285	0.44	0.4472	1.098
138	20	4.38	4.394	0.47	0.4472	-0.1406
1,363	13	5.05	5.05	0.5	0.4472	0.002993

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	24.56111	5	-39.12222
A2	24.8146	8	-33.6292
A3	24.56111	5	-39.12222
R	10.7627	2	-17.5254
4	23.46704	4	-38.93407

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	28.1	6	<0.0001
Test 2	0.507	3	0.9174
Test 3	0.507	3	0.9174
Test 6a	2.188	1	0.1391

Table 3-10. Summary of BMD modeling results for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation (Ema et al., 2008); BMR = 10% RD from control mean and 1 SD change from control mean

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3) ^b	0.235	-45.537	563	482	587	488	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M4)	0.882	-46.411	215	116	227	125	
Exponential (M5)	N/A ^c	-44.433	200	116	218	125	
Hill	N/A ^c	-44.433	207	112	223	120	
Power ^d Polynomial 3 ^o ^e Polynomial 2 ^o ^f Linear	0.278	-45.874	522	438	540	441	

^aConstant variance case presented. Both constant variance assumption and modeled variance were not appropriate in this case: BMDs Tests 2 and 3 with constant variance assumption rejected the null hypothesis with p-value = 0.00438; Test 3 of modeled variance also rejected the null hypothesis. A sensitivity analysis (see below) indicated limited effect of variance on model fitting. Selected model in bold; scaled residuals for selected model for doses 0, 14.7, 139.3, and 1,360 mg/kg-day were 0.09694, -0.1119, 0.01719, and -0.0007502, respectively.

^bFor the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

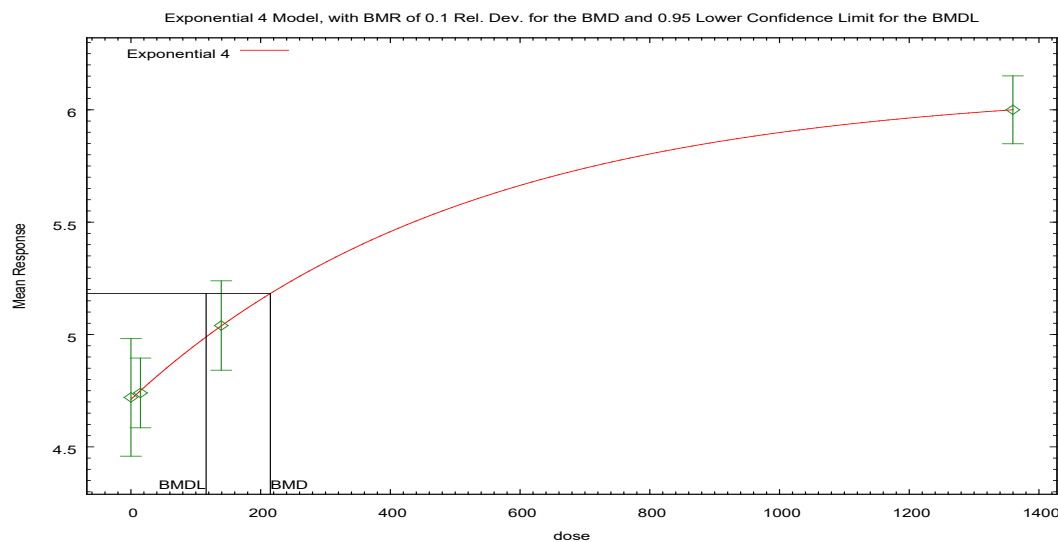
^cNo available degrees of freedom to calculate a goodness-of-fit value.

^dFor the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

^eFor the Polynomial 3^o model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

^fFor the Polynomial 2^o model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from [Ema et al. \(2008\)](#)



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure 3-19. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling male CRL

Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c-(c-1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 214.961

BMDL at the 95% confidence level = 115.944

Parameter Estimates

Variable	Estimate	Default initial parameter values
Lalpha	-1.72548	-1.72578
Rho	N/A	0
A	4.71128	4.484
B	0.00192508	0.00133871
C	1.29509	1.405
D	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.72	4.711	0.59	0.422	0.09694
14.7	22	4.74	4.75	0.35	0.422	-0.1119
139.3	18	5.04	5.038	0.4	0.422	0.01719
1,360	13	6	6	0.25	0.422	-0.0007502

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	27.21664	5	-44.43327
A2	33.77721	8	-51.55442
A3	27.21664	5	-44.43327
R	-2.570126	2	9.140253
4	27.20553	4	-46.41105

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	72.69	6	<0.0001
Test 2	13.12	3	0.004382

Test 3	13.12	3	0.004382
Test 6a	0.02222	1	0.8815

Sensitivity analysis:

The fit to the means was adequate for Exponential M4 with constant variance, and their scaled residuals were small. However, Tests 2 and 3 rejected the null hypothesis with both constant variance assumption and modeled variance, indicating lack of fit to variances whether the variance was constant or modeled as a power of the means. To determine how much BMDL_{10%RD} (116 mg/kg-day) was affected by the variance used, a sensitivity analysis was performed with constant variance by setting the standard deviation for all dose groups to the minimum or maximum observed values (0.25 and 0.59). Because the means were not changed and the constant-variance option was used, the parameters (including BMD) were unchanged. BMDLs (low confidence limit of BMD, BMR = 10% RD) were 147 mg/kg-day (with minimum standard deviation) and 96.7 mg/kg-day (with maximum standard deviation); the BMDLs were within twofold, suggesting limited effect of variance in this case. Therefore, the M4 model with constant variance was used to derive the BMD and BMDL for this data set.

Table 3-11. Sensitivity analysis with minimum SD as variance: Summary of BMD modeling results for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation ([Ema et al., 2008](#)); BMR = 10% RD from control mean

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3) ^b	0.0150	-122.66	563	512	
Exponential (M4)	0.796	-128.99	215	147	
Exponential (M5)	N/A ^c	-127.05	200	147	
Hill	N/A ^c	-127.05	207	148	
Power ^d Polynomial 3 ^o ^e Polynomial 2 ^o ^f Linear	0.0241	-123.60	522	468	

^aConstant variance case presented (BMDS Test 2 p-value = 1.000), selected model in bold; scaled residuals for selected model for doses 0, 14.7, 139.3, and 1,360 mg/kg-day were 0.1681, -0.1941, 0.02981, and -0.001301, respectively.

^bFor the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

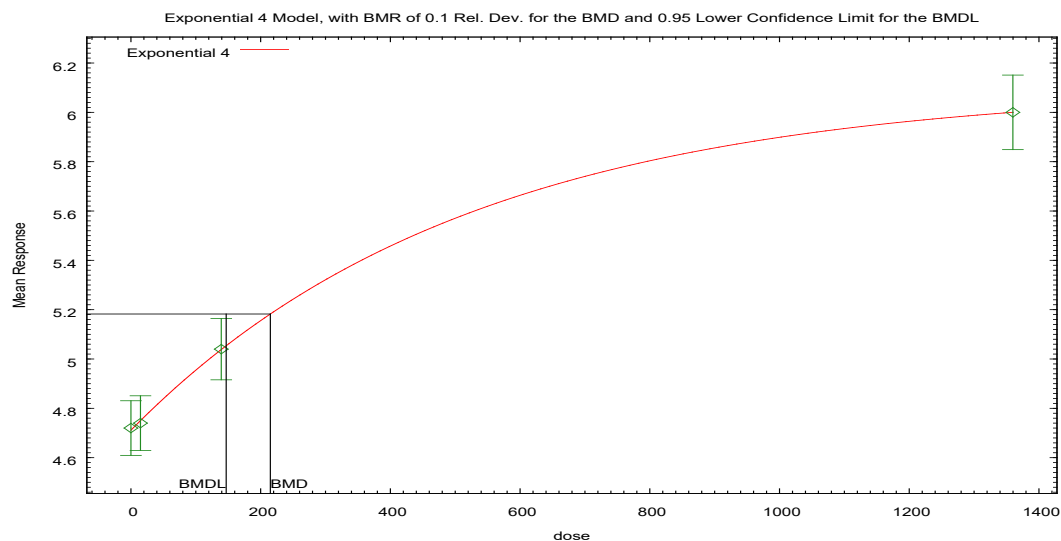
^cNo available degrees of freedom to calculate a goodness-of-fit value.

^dFor the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

^eFor the Polynomial 3^o model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

^fFor the Polynomial 2^o model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from [Ema et al. \(2008\)](#)



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure 3-20. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD during gestation and lactation on GD 0–PND 26, dose TWA gestation and lactation ([Ema et al., 2008](#)).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 214.961

BMDL at the 95% confidence level = 146.85

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-2.82651	-2.8274
rho	N/A	0
a	4.71128	4.484
b	0.00192508	0.00133871
c	1.29509	1.405
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
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0	22	4.72	4.711	0.25	0.2434	0.1681
14.7	22	4.74	4.75	0.25	0.2434	-0.1941
139.3	18	5.04	5.038	0.25	0.2434	0.02981
1,360	13	6	6	0.25	0.2434	-0.001301

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	68.52739	5	-127.0548
A2	68.53022	8	-121.0604
A3	68.52739	5	-127.0548
R	10.89708	2	-17.79415
4	68.49396	4	-128.9879

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	115.3	6	<0.0001
Test 2	0.00567	3	0.9999
Test 3	0.00567	3	0.9999
Test 6a	0.06685	1	0.796

Table D-3-12. Sensitivity analysis with maximum SD as variance: Summary of BMD modeling results for relative liver weight (g/10 0g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD by gestation and lactation on GD 0–PND 26, dose TWA gestation and lactation (Ema et al., 2008); BMR = 10% RD from control mean

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3) ^b	0.454	-0.67698	563	459	
Exponential (M4)	0.913	-0.24352	215	96.7	
Exponential (M5)	N/A ^c	1.7445	200	96.9	
Hill	N/A ^c	1.7445	207	90.2	
Power ^d Polynomial 3 ^{oe} Polynomial 2 ^{of} Linear	0.498	-0.86210	522	414	

^aConstant variance case presented (BMDS Test 2 p-value = 1.000), selected model in bold; scaled residuals for selected model for doses 0, 14.7, 139.3, and 1,360 mg/kg-day were 0.07126, -0.08227, 0.01264, and -0.0005523, respectively.

^bFor the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

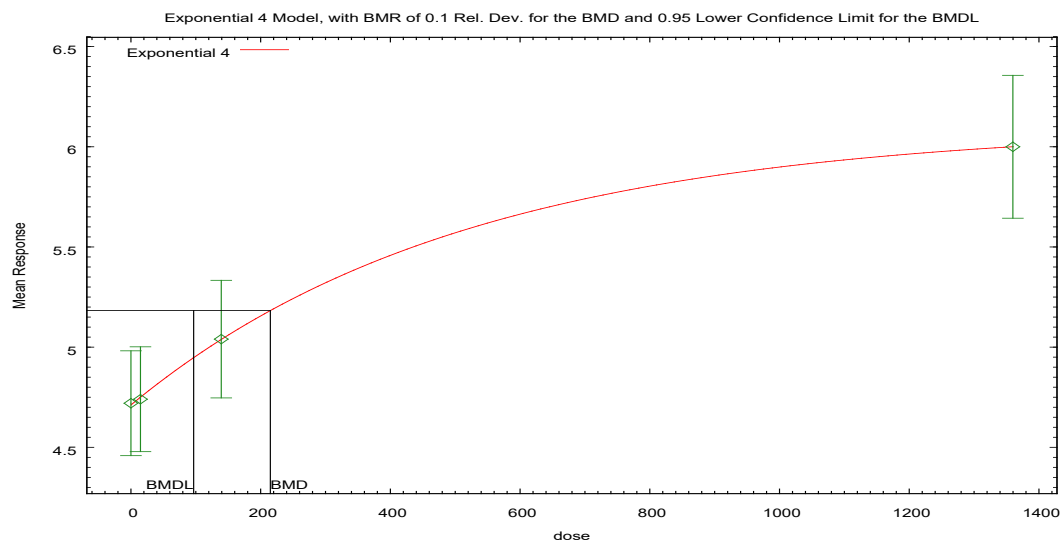
^cNo available degrees of freedom to calculate a goodness-of-fit value.

^dFor the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

^eFor the Polynomial 3^o model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

^fFor the Polynomial 2^o model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from [Ema et al. \(2008\)](#)



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure 3-21. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation ([Ema et al., 2008](#)).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 214.962

BMDL at the 95% confidence level = 96.7112

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-1.10991	-1.11007
rho	N/A	0
a	4.71128	4.484
b	0.00192507	0.00133871
c	1.29509	1.405

d	N/A	1
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Table of Data and Estimated Values of Interest

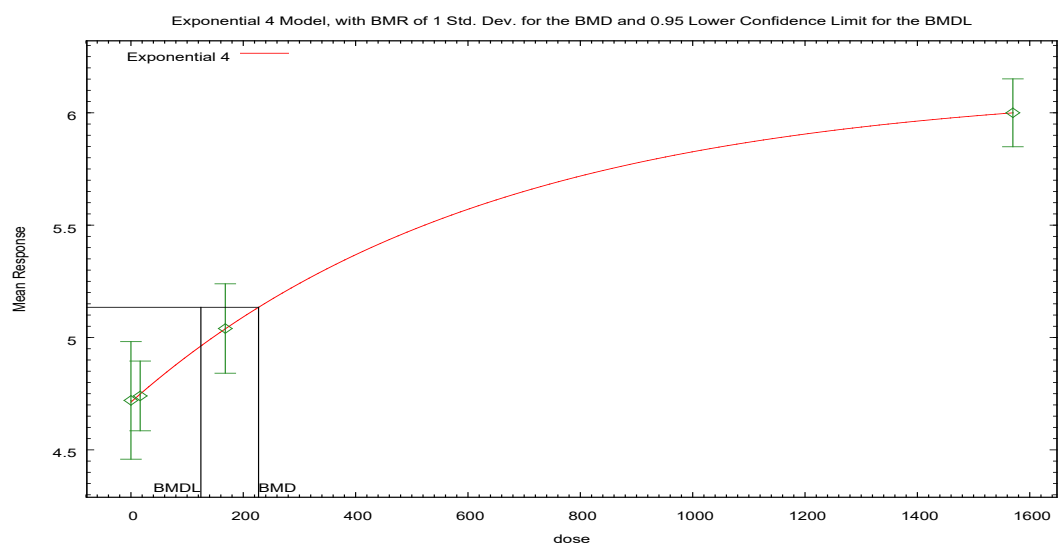
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.72	4.711	0.59	0.5741	0.07126
14.7	22	4.74	4.75	0.59	0.5741	-0.08227
139.3	18	5.04	5.038	0.59	0.5741	0.01264
1,360	13	6	6	0.59	0.5741	-0.0005523

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	4.127765	5	1.744471
A2	4.130599	8	7.738801
A3	4.127765	5	1.744471
R	-14.77144	2	33.54287
4	4.121761	4	-0.2435229

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	37.8	6	<0.0001
Test 2	0.00567	3	0.9999
Test 3	0.00567	3	0.9999
Test 6a	0.01201	1	0.9127



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure 3-22. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 227.183

BMDL at the 95% confidence level = 124.503

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-1.72556	-1.72578
rho	N/A	0
a	4.71255	4.484
b	0.00156899	0.00115941
c	1.29864	1.405
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.72	4.713	0.59	0.422	0.08283
16.5	22	4.74	4.749	0.35	0.422	-0.09464
168	18	5.04	5.039	0.4	0.422	0.01356
1,570	13	6	6	0.25	0.422	-0.0006035

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	27.21664	5	-44.43327
A2	33.77721	8	-51.55442
A3	27.21664	5	-44.43327
R	-2.570126	2	9.140253
4	27.20864	4	-46.41727

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
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Test 1	72.69	6	<0.0001
Test 2	13.12	3	0.004382
Test 3	13.12	3	0.004382
Test 6a	0.016	1	0.8993

Table 3-13. Summary of BMD modeling results for relative liver weight (g/100 g BW) in F2 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose as TWA of gestation and lactation (Ema et al., 2008); BMR = 10% RD from control mean and 1 SD change from control mean

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3) ^b	0.265	-92.639	589	520	400	339	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M4)	0.759	-93.205	286	166	177	103	
Exponential (M5)	N/A ^c	-91.299	168	141	149	104	
Hill	N/A ^c	-91.299	153	error ^d	144	101	
Power ^e Polynomial 3 ^{of} Polynomial 2 ^{og} Linear	0.323	-93.039	549	477	367	307	

^aConstant variance case presented (BMDS Test 2 p-value = 0.192), selected model in bold; scaled residuals for selected model for doses 0, 14.7, 139.3, and 1,360 mg/kg-day were 0.2031, -0.2277, 0.03152, and -0.001049, respectively.

^bFor the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

^cNo available degrees of freedom to calculate a goodness-of-fit value.

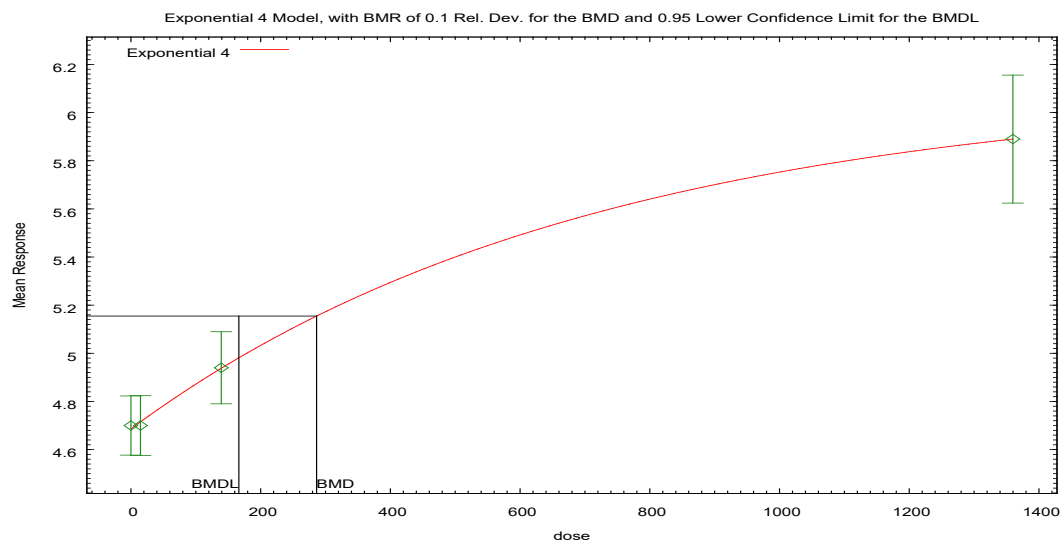
^dBMD or BMDL computation failed for this model.

^eFor the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

^fFor the Polynomial 3^o model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space) The models in this row reduced to the Linear model.

^gFor the Polynomial 2^o model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from [Ema et al. \(2008\)](#)



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure 3-23. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose as TWA of gestation and lactation (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 286.259

BMDL at the 95% confidence level = 166.437

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-2.33164	-2.33288
rho	N/A	0
a	4.68619	4.465
b	0.00140932	0.00130926
c	1.30123	1.38511
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
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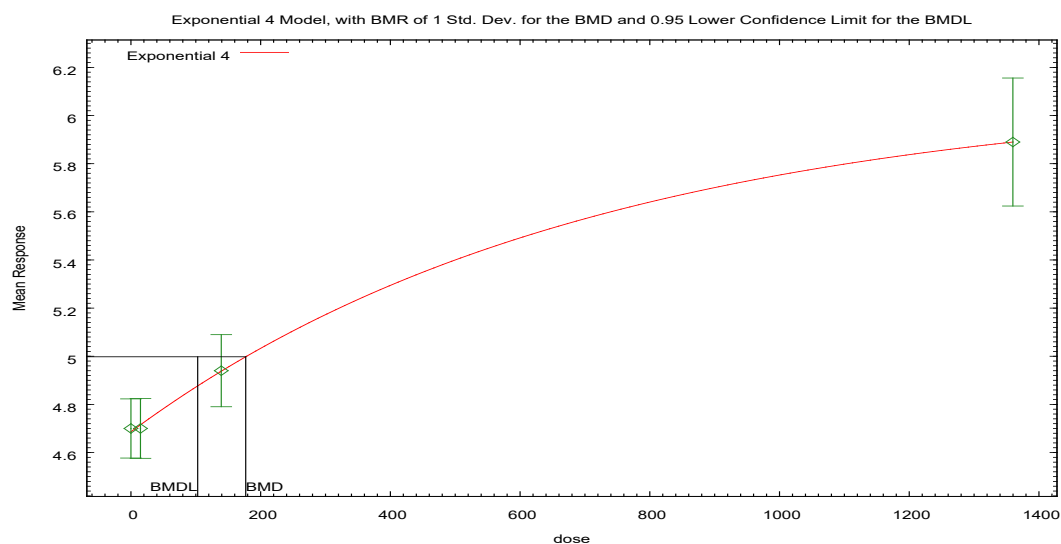
0	21	4.7	4.686	0.27	0.3117	0.2031
14.7	22	4.7	4.715	0.28	0.3117	-0.2277
139.3	20	4.94	4.938	0.32	0.3117	0.03152
1,360	13	5.89	5.89	0.44	0.3117	-0.001049

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	50.6495	5	-91.299
A2	53.0199	8	-90.03981
A3	50.6495	5	-91.299
R	9.931909	2	-15.86382
4	50.60242	4	-93.20485

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	86.18	6	<0.0001
Test 2	4.741	3	0.1918
Test 3	4.741	3	0.1918
Test 6a	0.09415	1	0.759



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure 3-24. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose as TWA of gestation and lactation (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 177.017

BMDL at the 95% confidence level = 102.961

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	-2.33164	-2.33288
rho	N/A	0
a	4.68619	4.465
b	0.00140932	0.00130926
c	1.30123	1.38511
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	4.7	4.686	0.27	0.3117	0.2031
14.7	22	4.7	4.715	0.28	0.3117	-0.2277
139.3	20	4.94	4.938	0.32	0.3117	0.03152
1,360	13	5.89	5.89	0.44	0.3117	-0.001049

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	50.6495	5	-91.299
A2	53.0199	8	-90.03981
A3	50.6495	5	-91.299
R	9.931909	2	-15.86382
4	50.60242	4	-93.20485

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	86.18	6	<0.0001
Test 2	4.741	3	0.1918
Test 3	4.741	3	0.1918
Test 6a	0.09415	1	0.759

Table 3-14. Summary of BMD modeling results for relative liver weight (g/100 g BW) in male CRL Sprague-Dawley rats exposed to HBCD by gavage for 13 weeks ([WIL Research, 2001](#)); BMR = 10% RD from control mean and 1 SD change from control mean

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Modeled with constant variance							No model showed adequate fit. Dropping highest dose is not expected to help in this case.
Exponential (M2) Exponential (M3) ^b	3.14 × 10 ⁻⁴	-67.830	328	283	269	219	
Exponential (M4) ^c	3.92 × 10 ⁻⁴	-69.396	164	97.7	128	77.9	
Exponential (M5) ^d	3.92 × 10 ⁻⁴	-69.396	164	97.7	128	77.9	
Hill	4.91 × 10 ⁻⁴	-69.815	145	74.8	113	59.7	
Power ^e Polynomial 3 ^{of} Polynomial 2 ^{og} Linear	5.14 × 10 ⁻⁴	-68.817	290	244	234	187	
Modeled with modeled variance							
Exponential (M2) Exponential (M3) ^b	0.00119	-68.721	337	295	320	245	
Exponential (M4) ^c	5.50 × 10 ⁻⁴	-68.244	204	103	187	67.5	
Exponential (M5) ^d	5.50 × 10 ⁻⁴	-68.244	204	103	187	67.5	
Hill	5.84 × 10 ⁻⁴	-68.355	192	35.9	173	106	
Power ^e Polynomial 3 ^{of} Polynomial 2 ^{og} Linear	0.00161	-69.324	299	256	282	210	

^aConstant variance (BMDS Test 2 p-value = 0.0644, BMDS Test 3 p-value = 0.0644) and nonconstant variance cases presented, no model was selected as a best-fitting model.

^bFor the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

^cThe Exponential (M4) model may appear equivalent to the Exponential (M5) model; however, differences exist in digits not displayed in the table.

^dThe Exponential (M5) model may appear equivalent to the Exponential (M4) model; however, differences exist in digits not displayed in the table.

^eFor the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

^fFor the Polynomial 3^o model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

^gFor the Polynomial 2^o model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from [WIL Research \(2001\)](#)

Table 3-15. Summary of BMD modeling results for relative liver weight (g/100 g BW) in female CRL Sprague-Dawley rats exposed to HBCD by gavage for 13 weeks ([WIL Research, 2001](#)); BMR = 10% RD from control mean and 1 SD change from control mean

Model ^a	Goodness of fit		BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Modeled with constant variance							No model showed adequate fit. Dropping highest dose is not expected to help in this case
Exponential (M2) Exponential (M3) ^b	<0.0001	-39.545	310	261	332	267	
Exponential (M4) Exponential (M5) ^c	2.59 × 10 ⁻⁴	-44.035	101	56.0	106	61.8	
Hill	5.71 × 10 ⁻⁴	-45.515	69.3	30.6	73.3	34.6	
Power ^d Polynomial 3 ^{oe} Polynomial 2 ^{of} Linear	<0.0001	-40.679	270	220	287	226	
Modeled with modeled variance							
Exponential (M2) Exponential (M3) ^b	<0.0001	-38.793	319	269	374	282	
Exponential (M4) Exponential (M5) ^c	1.72 × 10 ⁻⁴	-42.217	53.4	28.5	38.3	16.0	
Hill	0.00115	-45.763	39.2	20.7	26.0	11.6	
Power Polynomial 3 ^{oe} Polynomial 2 ^{of} Linear	<0.0001	-39.727	278	227	327	237	

^aConstant variance (BMDS Test 2 p-value = 0.461, BMDS Test 3 p-value = 0.461) and nonconstant variance presented; no model was selected as a best-fitting model.

^bFor the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

^cFor the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.

^dFor the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

^eFor the Polynomial 3^o model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

^fFor the Polynomial 2^o model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

3.2.3.3 Reproductive

Table 3-16. Summary of BMD modeling results for primordial follicles in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008); BMR = 1% RD from control mean, 5% RD from control mean, and 10% RD from control mean

Modela	Goodness of fit		BMD _{1RD} (mg/kg-d)	BMDL _{1RD} (mg/kg-d)	BMD _{5RD} (mg/kg-d)	BMDL _{5RD} (mg/kg-d)	BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	Basis for model selection
	p-value	AIC							
Exponential (M2) Exponential (M3) ^b	0.0130	408.57	26.8	13.9	137	71.0	281	146	Exponential M4 constant variance selected as only model with adequate fit.
Exponential (M4)	0.688	402.05	0.883	0.252	4.67	1.33	10.1	2.87	
Exponential (M5)	N/A ^c	403.91	4.09	0.259	8.23	1.37	11.4	2.95	
Hill	N/A ^c	403.91	8.00	error ^d	9.28	1.10	9.99	2.50	
Power ^e Polynomial 2 ^{of} Linear Polynomial 3 ^{og}	0.0117	408.78	33.1	19.8	165	99.0	331	198	

^aConstant variance case presented (BMDS Test 2 p-value = 0.242), selected model in bold; scaled residuals for selected model for doses 0, 9.6, 96.3, and 940.7 mg/kg-day were -0.129, 0.1915, -0.2611, and 0.1987, respectively.

^bFor the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

^cNo available degrees of freedom to calculate a goodness-of-fit value.

^dBMD or BMDL computation failed for this model.

^eFor the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

^fFor the Polynomial 2^o model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

^gThe Polynomial 3^o model may appear equivalent to the Linear model; however, differences exist in digits not displayed in the table.

Data from [Ema et al. \(2008\)](#)

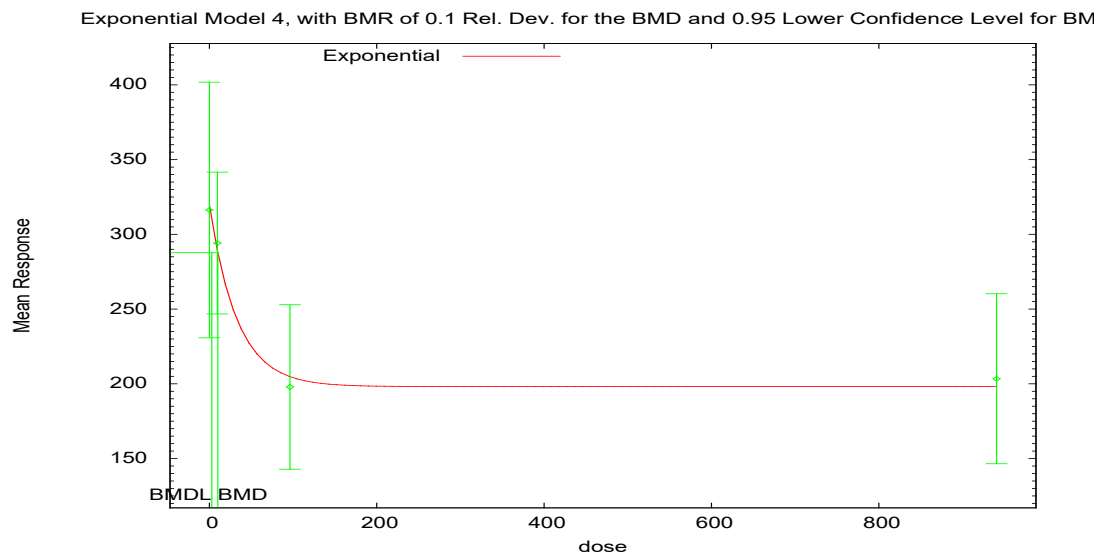


Figure 3-25. Plot of mean response by dose, with fitted curve for Exponential M4, for primordial follicles in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks ([Ema et al., 2008](#)).

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 10.1143

BMDL at the 95% confidence level = 2.86589

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	8.85121	8.84717
rho(S)	N/A	0
a	319.71	332.115
b	0.0301725	0.0026785
c	0.619779	0.567503
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	10	316.3	319.7	119.5	83.56	-0.129
9.6	10	294.2	289.1	66.3	83.56	0.1915

96.3	10	197.9	204.8	76.9	83.56	-0.2611
940.7	10	203.4	198.1	79.5	83.56	0.1987

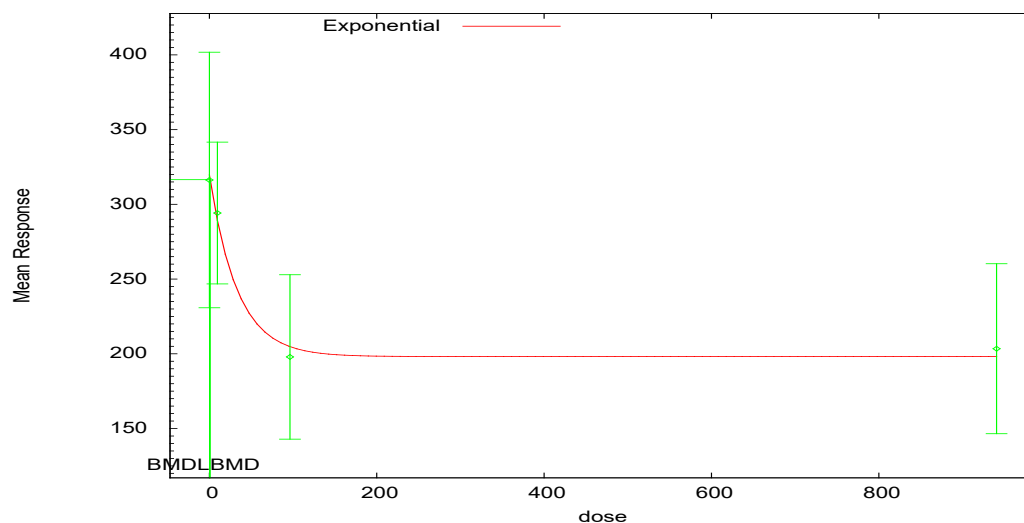
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-196.9435	5	403.8869
A2	-194.8505	8	405.701
A3	-196.9435	5	403.8869
R	-203.7104	2	411.4207
4	-197.0241	4	402.0483

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	17.72	6	0.006972
Test 2	4.186	3	0.2421
Test 3	4.186	3	0.2421
Test 6a	0.1613	1	0.6879

Exponential Model 4, with BMR of 0.01 Rel. Dev. for the BMD and 0.95 Lower Confidence Level for BA



12:46 02/11 2015
 BMR = 1% RD from control mean; dose shown in mg/kg-day.

Figure 3-26. Plot of mean response by dose, with fitted curve for Exponential M4, for primordial follicles in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: $Y[\text{dose}] = a * [c - (c-1) * \exp(-b * \text{dose})]$
 A constant variance model is fit

Benchmark Dose Computation

BMR = 1% RD

BMD = 0.883338

BMDL at the 95% confidence level = 0.251965

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	8.85121	8.84717
rho(S)	N/A	0
a	319.71	332.115
b	0.0301725	0.0026785
c	0.619779	0.567503
d	1	1

Table of Data and Estimated Values of Interest

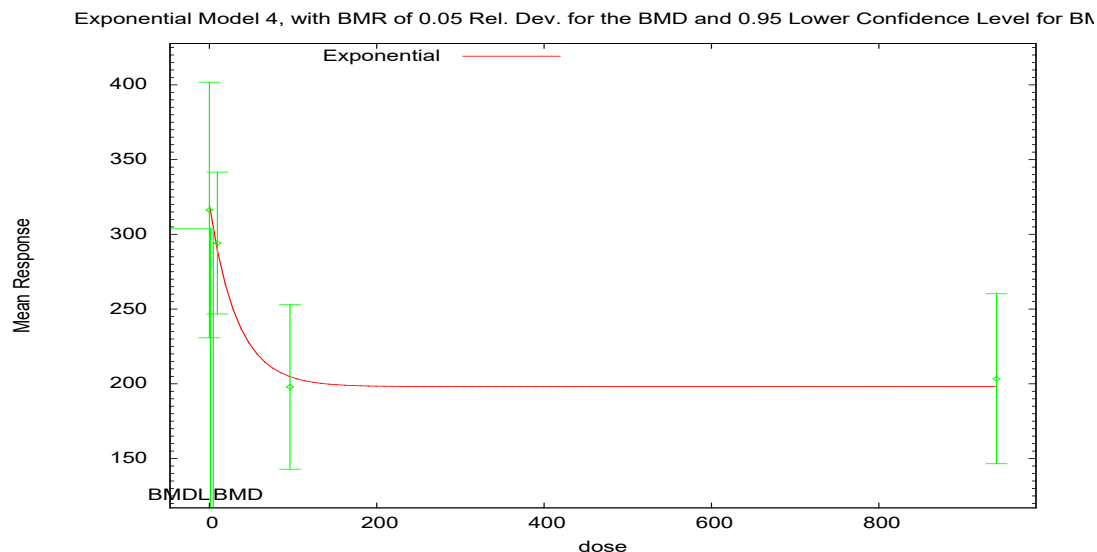
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	10	316.3	319.7	119.5	83.56	-0.129
9.6	10	294.2	289.1	66.3	83.56	0.1915
96.3	10	197.9	204.8	76.9	83.56	-0.2611
940.7	10	203.4	198.1	79.5	83.56	0.1987

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-196.9435	5	403.8869
A2	-194.8505	8	405.701
A3	-196.9435	5	403.8869
R	-203.7104	2	411.4207
4	-197.0241	4	402.0483

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	17.72	6	0.006972
Test 2	4.186	3	0.2421
Test 3	4.186	3	0.2421
Test 6a	0.1613	1	0.6879



BMR = 5% RD from control mean; dose shown in mg/kg-day.

Figure 3-27. Plot of mean response by dose, with fitted curve for Exponential Model 4, for primordial follicles in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks (Ema et al., 2008).

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 5% RD

BMD = 4.67281

BMDL at the 95% confidence level = 1.32975

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	8.85121	8.84717
rho(S)	N/A	0
a	319.71	332.115
b	0.0301725	0.0026785
c	0.619779	0.567503
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	10	316.3	319.7	119.5	83.56	-0.129
9.6	10	294.2	289.1	66.3	83.56	0.1915

96.3	10	197.9	204.8	76.9	83.56	-0.2611
940.7	10	203.4	198.1	79.5	83.56	0.1987

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-196.9435	5	403.8869
A2	-194.8505	8	405.701
A3	-196.9435	5	403.8869
R	-203.7104	2	411.4207
4	-197.0241	4	402.0483

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	17.72	6	0.006972
Test 2	4.186	3	0.2421
Test 3	4.186	3	0.2421
Test 6a	0.1613	1	0.6879

Data from [Ema et al. \(2008\)](#) for incidence of non-pregnancy.

Table 3-17. Summary of BMD modeling results for incidence of non-pregnancy in F0 and F1 CRL female rats combined exposed to HBCD in diet for 14 weeks, TWA F0 and F1 pre-mating dose ([Ema et al., 2008](#)); BMR = 5% ER and 10% ER

Model ^a	Goodness of fit		BMD _{5Pct} (mg/kg-d)	BMDL _{5Pct} (mg/kg-d)	BMD _{10Pct} (mg/kg-d)	BMDL _{10Pct} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Gamma Weibull Multistage 3° Multistage 2° Quantal-Linear	0.0881	120.47	617	263	1,266	541	No models provided an adequate fit and a valid BMDL estimate; therefore no model was selected.
Dichotomous-Hill	N/A ^b	119.61	15.1	error ^c	35.8	13.4	
Logistic	0.0806	120.75	824	482	1,401	817	
LogLogistic	0.0897	120.43	584	230	1,232	486	
Probit	0.0815	120.72	797	449	1,392	781	
LogProbit	0.396	118.31	6.18	error ^c	159	error ^c	

^aNo model was selected as a best-fitting model.

^bNo available degrees of freedom to calculate a goodness-of-fit value.

^cBMD or BMDL computation failed for this model.

Table 3-18. Summary of BMD modeling results for incidence of non-pregnancy in F0 and F1 CRL female rats combined exposed to HBCD in diet for 14 weeks, TWA F0 and F1 pre-mating dose, high dose dropped (Ema et al., 2008); BMR = 5% ER and 10% ER.

Model ^a	Goodness of fit		BMD _{5Pct} (mg/kg-d)	BMDL _{5Pct} (mg/kg-d)	BMD _{10Pct} (mg/kg-d)	BMDL _{10Pct} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Gamma ^b	0.457	76.591	51.1	25.6	105	52.5	Of the models that provided an adequate fit and a valid BMDL estimate, the LogLogistic model was selected based on lowest AIC.
Logistic	0.374	76.860	77.3	53.3	121	85.5	
LogLogistic	0.469	76.560	48.5	22.7	102	47.9	
Probit	0.382	76.832	73.6	49.3	120	81.1	
LogProbit	N/A ^c	78.045	18.0	error ^d	74.8	error ^d	
Weibull ^e	0.457	76.591	51.1	25.6	105	52.5	
Quantal-Linear ^f	0.457	76.591	51.1	25.6	105	52.5	
Multistage 2 ^o ^g	0.457	76.591	51.1	25.6	105	52.5	

^aSelected model in bold; scaled residuals for selected model for doses 0, 13.3, and 131.5 mg/kg-day were -0.422, 0.575, and -0.128, respectively.

^bThe Gamma model may appear equivalent to the Weibull model; however, differences exist in digits not displayed in the table. This also applies to the Multistage 2^o and Quantal-Linear models.

^cNo available degrees of freedom to calculate a goodness-of-fit value.

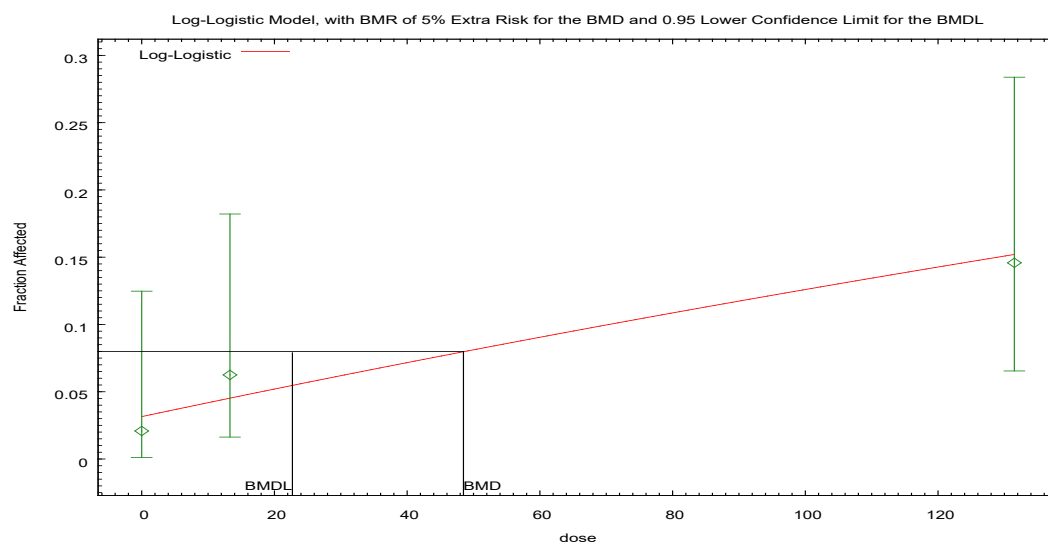
^dBMD or BMDL computation failed for this model.

^eFor the Weibull model, the power parameter estimate was 1. The models in this row reduced to the Quantal-Linear model.

^fThe Quantal-Linear model may appear equivalent to the Gamma model; however, differences exist in digits not displayed in the table. This also applies to the Multistage 2^o model.

^gThe Multistage 2^o model may appear equivalent to the Gamma model; however, differences exist in digits not displayed in the table. This also applies to the Weibull and Quantal-Linear models.

Data from [Ema et al. \(2008\)](#)



BMR = 5% ER; dose shown in mg/kg-day.

Figure 3-28. Plot of incidence rate by dose with fitted curve for LogLogistic model for incidence of non-pregnancy in F0 and F1 CRL female rats combined exposed to HBCD in diet for 14 weeks, TWA F0 and F1 pre-mating dose, high dose dropped (Ema et al., 2008).

Logistic Model (Version: 2.14; Date: 2/28/2013)

The form of the probability function is: $P[\text{response}] = \text{background} + (1 - \text{background}) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))]$

Slope parameter is restricted as slope ≥ 1

Benchmark Dose Computation

BMR = 5% ER

BMD = 48.4809

BMDL at the 95% confidence level = 22.7093

Parameter Estimates

Variable	Estimate	Default initial parameter values
background	0.0314626	0.0208333
intercept	-6.8256E+00	-6.4682E+00
slope	1	1

Analysis of Deviance Table

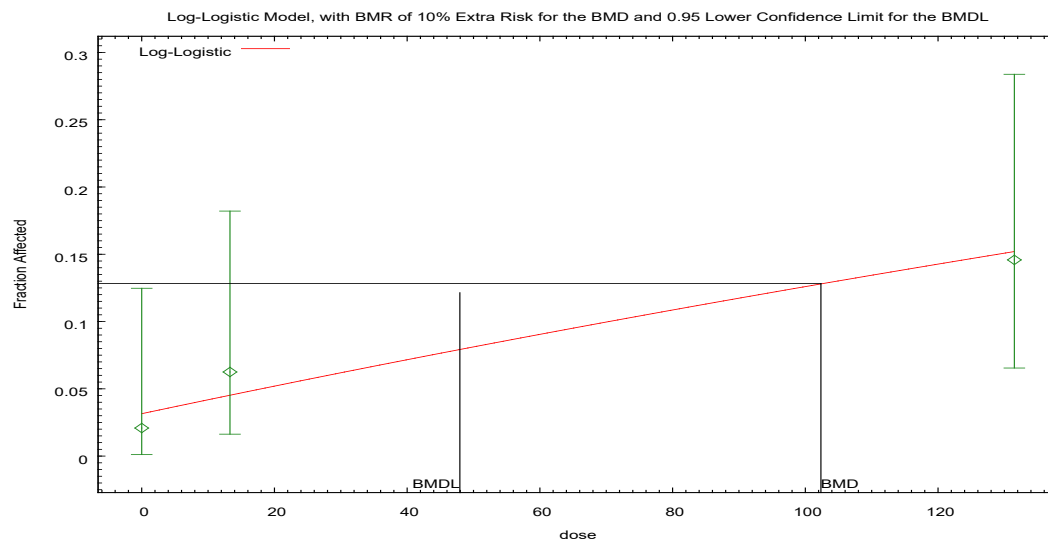
Model	Log (likelihood)	Number of parameters	Deviance	Test df	p-value
Full model	-36.0225	3			
Fitted model	-36.28	2	0.514904	1	0.473
Reduced model	-38.8598	1	5.6746	2	0.05858

AIC: = 76.56

Goodness-of-Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled residuals
0	0.0315	1.51	1	48	-0.422
13.3	0.0452	2.172	3	48	0.575
131.5	0.1525	7.318	7	48	-0.128

Chi² = 0.52, df = 1, p-value = 0.4687



22:27 05/20 2016
 BMR = 10% ER; dose shown in mg/kg-day.

Figure 3-29. Plot of incidence rate by dose with fitted curve for LogLogistic model for incidence of non-pregnancy in F0 and F1 CRL female rats combined exposed to HBCD in diet for 14 weeks, TWA F0 and F1 pre-mating dose, high dose dropped (Ema et al., 2008).

Logistic Model (Version: 2.14; Date: 2/28/2013)

The form of the probability function is: $P[\text{response}] = \text{background} + (1 - \text{background}) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))]$

Slope parameter is restricted as slope ≥ 1

Benchmark Dose Computation

BMR = 10% ER
 BMD = 102.349
 BMDL at the 95% confidence level = 47.9419

Parameter Estimates

Variable	Estimate	Default initial parameter values
background	0.0314626	0.0208333
intercept	-6.8256E+00	-6.4682E+00
slope	1	1

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test df	p-value
Full model	-36.0225	3			
Fitted model	-36.28	2	0.514904	1	0.473
Reduced model	-38.8598	1	5.6746	2	0.05858

AIC: = 76.56

Goodness-of-Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled residuals
0	0.0315	1.51	1	48	-0.422
13.3	0.0452	2.172	3	48	0.575
131.5	0.1525	7.318	7	48	-0.128

Chi² = 0.52, df = 1, p-value = 0.4687

3.2.3.4 Developmental

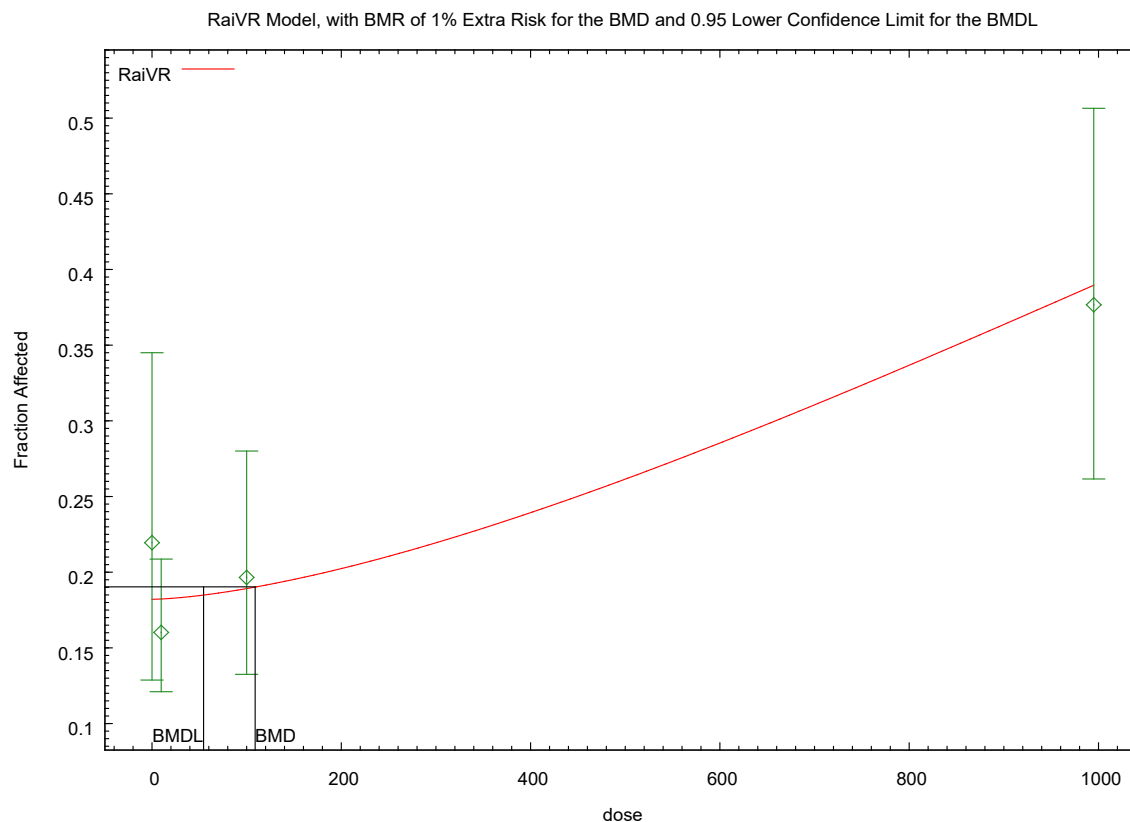
Table 3-19. Summary of BMD modeling results for offspring loss from implantation through PND 4 in F2 offspring CRL Sprague-Dawley rats; gestational doses of F1 dams (Ema et al., 2008); BMR = 1% ER and 5% ER

Model ^a	Goodness of Fit		BMD _{1Pct} (mg/kg-d)	BMDL _{1Pct} (mg/kg-d)	BMD _{5Pct} (mg/kg-d)	BMDL _{5Pct} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Litter-specific covariate = implantation size; intra-litter correlations estimated							Of the models that provided an adequate fit, a valid BMDL estimate and BMD/BMDL <5, the NCTR/Rai and Van Ryzin model (litter-specific covariate not used; intra-litter correlations estimated) was selected based on lowest BMDL (BMDLs differed by >3).
Nested Logistic	0.1776	1,236.98	523.682	17.8051	708.771	92.7735	
NCTR	0.1770	1,237.29	450.409	225.409	659.055	329.826	
Rai and Van Ryzin	0.1984	1,236.26	371.593	185.81	538.091	269.046	
Litter-specific covariate = implantation size; intra-litter correlations assumed to be zero							
Nested Logistic	0.0000	1,337.62	560.759	26.8162	740.805	139.727	
NCTR	0.0000	1,335.98	553.123	460.936	739.356	616.13	
Rai and Van Ryzin	0.0000	1,337.63	138.735	86.7096	291.342	291.342	
Litter-specific covariate not used; intra-litter correlations estimated							
Nested Logistic	0.1377	1,234.32	105.863	17.0526	301.093	88.853	
NCTR ^b Rai and Van Ryzin	0.1423	1,234.32	108.957	54.4786	315.584	157.792	
Litter-specific covariate not used; intra-litter correlations assumed to be zero							
Nested Logistic	0.0000	1,336.56	132.255	25.2574	353.37	131.605	
NCTR ^b Rai and Van Ryzin	0.0000	1,336.56	136.105	68.0523	367.95	183.975	

^aBecause the individual animal data were available, the BMDS nested models were fitted, with the selected model in bold. For the selected model, the proportion of litters with scaled residuals above 2 in absolute value for doses 0, 9.7, 100, and 995 mg/kg-day were 2/23, 1/23, 1/20, and 1/21, respectively.

^bWith the litter-specific covariate not used, the NCTR and Rai and van Ryzin models yielded identical results.

Data from [Ema et al. \(2008\)](#)



15:15 08/09 2016

BMR = 1% ER; dose shown in mg/kg-day.

Figure 3-30. Plot of incidence rate by dose, with fitted curve for the nested Rai and Van Ryzin model where the litter specific covariate was not used and the intra-litter correlations were estimated, for incidence of offspring loss from implantation through PND 4 in F2 offspring CRL Sprague-Dawley rats; gestational doses of F1 dams ([Ema et al., 2008](#)).

Rai and Van Ryzin Model (Version: 2.12; Date: 04/27/2015)

The form of the probability function is:

$$\text{Prob.} = [1 - \exp(-\text{Alpha} - \text{Beta} * \text{Dose}^{\text{Rho}})] * \exp(-(\text{Th1} + \text{Th2} * \text{Dose}) * \text{Rij}),$$

where Rij is the litter specific covariate.

Restrict Power rho >= 1.

Benchmark Dose Computation

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all the data: 14.425287

BMR = 1% ER

BMD = 108.957

BMDL at the 95% confidence level = 54.4787

Parameter Estimates

Variable	Estimate	(Default) Initial Parameter Values
alpha	0.201085	0.201085
beta	7.58104×10^{-6}	7.58104×10^{-6}
rho	1.53267	1.53267
phi1	0.222343	0.222343
phi2	0.0213907	0.0213907
phi3	0.0759418	0.0759418
phi4	0.277171	0.277171

Log-likelihood: -610.162 AIC: 1,234.32

Goodness-of-Fit Table

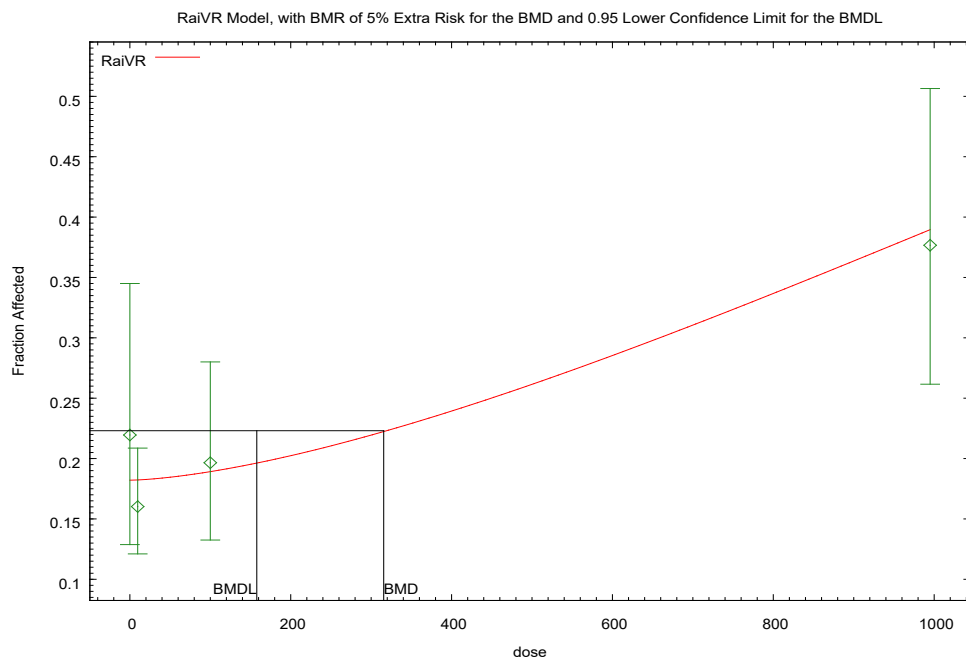
Dose	Lit.-Spec.		Litter		Scaled		Residual
	Cov.	Est.	Prob.	Size	Expected	Observed	
0.0000	9.0000	0.182	9	1.639	3	0.7049	
0.0000	10.0000	0.182	10	1.822	4	1.0303	
0.0000	11.0000	0.182	11	2.004	5	1.3037	
0.0000	11.0000	0.182	11	2.004	0	-0.8718	
0.0000	12.0000	0.182	12	2.186	1	-0.4778	
0.0000	13.0000	0.182	13	2.368	0	-0.8885	
0.0000	13.0000	0.182	13	2.368	3	0.2371	
0.0000	13.0000	0.182	13	2.368	3	0.2371	
0.0000	13.0000	0.182	13	2.368	0	-0.8885	
0.0000	14.0000	0.182	14	2.550	1	-0.5442	
0.0000	14.0000	0.182	14	2.550	3	0.1579	
0.0000	15.0000	0.182	15	2.732	15	4.0466	
0.0000	15.0000	0.182	15	2.732	11	2.7271	
0.0000	16.0000	0.182	16	2.915	4	0.3377	
0.0000	16.0000	0.182	16	2.915	2	-0.2845	
0.0000	16.0000	0.182	16	2.915	2	-0.2845	
0.0000	16.0000	0.182	16	2.915	1	-0.5956	
0.0000	16.0000	0.182	16	2.915	2	-0.2845	
0.0000	16.0000	0.182	16	2.915	2	-0.2845	
0.0000	17.0000	0.182	17	3.097	3	-0.0285	
0.0000	17.0000	0.182	17	3.097	0	-0.9115	
0.0000	17.0000	0.182	17	3.097	6	0.8546	
0.0000	18.0000	0.182	18	3.279	1	-0.6365	
9.7000	2.0000	0.182	2	0.365	2	2.9630	
9.7000	12.0000	0.182	12	2.188	5	1.8912	
9.7000	13.0000	0.182	13	2.371	3	0.4032	
9.7000	13.0000	0.182	13	2.371	0	-1.5189	
9.7000	13.0000	0.182	13	2.371	4	1.0439	
9.7000	14.0000	0.182	14	2.553	3	0.2736	
9.7000	14.0000	0.182	14	2.553	1	-0.9508	
9.7000	14.0000	0.182	14	2.553	1	-0.9508	
9.7000	14.0000	0.182	14	2.553	0	-1.5630	
9.7000	14.0000	0.182	14	2.553	2	-0.3386	
9.7000	15.0000	0.182	15	2.735	4	0.7418	
9.7000	15.0000	0.182	15	2.735	4	0.7418	
9.7000	15.0000	0.182	15	2.735	3	0.1552	

9.7000	15.0000	0.182	15	2.735	2	-0.4314
9.7000	16.0000	0.182	16	2.918	0	-1.6437
9.7000	16.0000	0.182	16	2.918	2	-0.5170
9.7000	16.0000	0.182	16	2.918	1	-1.0803
9.7000	16.0000	0.182	16	2.918	2	-0.5170
9.7000	17.0000	0.182	17	3.100	3	-0.0543
9.7000	17.0000	0.182	17	3.100	1	-1.1386
9.7000	17.0000	0.182	17	3.100	4	0.4879
9.7000	18.0000	0.182	18	3.282	3	-0.1476
9.7000	21.0000	0.182	21	3.830	4	0.0806

100.0000	11.0000	0.189	11	2.083	3	0.5323
100.0000	11.0000	0.189	11	2.083	1	-0.6282
100.0000	12.0000	0.189	12	2.272	0	-1.2357
100.0000	13.0000	0.189	13	2.461	0	-1.2604
100.0000	14.0000	0.189	14	2.651	2	-0.3149
100.0000	14.0000	0.189	14	2.651	3	0.1691
100.0000	14.0000	0.189	14	2.651	5	1.1369
100.0000	14.0000	0.189	14	2.651	2	-0.3149
100.0000	14.0000	0.189	14	2.651	6	1.6208
100.0000	14.0000	0.189	14	2.651	1	-0.7988
100.0000	14.0000	0.189	14	2.651	2	-0.3149
100.0000	15.0000	0.189	15	2.840	1	-0.8442
100.0000	15.0000	0.189	15	2.840	2	-0.3854
100.0000	15.0000	0.189	15	2.840	0	-1.3031
100.0000	15.0000	0.189	15	2.840	3	0.0734
100.0000	16.0000	0.189	16	3.029	4	0.4235
100.0000	16.0000	0.189	16	3.029	2	-0.4491
100.0000	17.0000	0.189	17	3.219	3	-0.0910
100.0000	17.0000	0.189	17	3.219	7	1.5729
100.0000	19.0000	0.189	19	3.597	10	2.4370

995.0000	7.0000	0.393	7	2.751	7	2.0149
995.0000	10.0000	0.393	10	3.930	2	-0.6684
995.0000	11.0000	0.393	11	4.323	3	-0.4205
995.0000	12.0000	0.393	12	4.716	0	-1.3852
995.0000	12.0000	0.393	12	4.716	6	0.3772
995.0000	13.0000	0.393	13	5.109	9	1.0623
995.0000	14.0000	0.393	14	5.502	4	-0.3831
995.0000	14.0000	0.393	14	5.502	0	-1.4032
995.0000	14.0000	0.393	14	5.502	2	-0.8932
995.0000	14.0000	0.393	14	5.502	10	1.1472
995.0000	15.0000	0.393	15	5.895	8	0.5037
995.0000	15.0000	0.393	15	5.895	3	-0.6928
995.0000	15.0000	0.393	15	5.895	9	0.7430
995.0000	15.0000	0.393	15	5.895	11	1.2216
995.0000	16.0000	0.393	16	6.288	15	1.9636
995.0000	16.0000	0.393	16	6.288	4	-0.5157
995.0000	16.0000	0.393	16	6.288	2	-0.9664
995.0000	17.0000	0.393	17	6.681	6	-0.1451
995.0000	17.0000	0.393	17	6.681	1	-1.2101
995.0000	17.0000	0.393	17	6.681	5	-0.3581
995.0000	20.0000	0.393	20	7.860	6	-0.3402

Observed Chi-square = 102.1763 Bootstrap Iterations per run = 10,000
p-value = 0.1423



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 BMR = 5% ER; dose shown in mg/kg-day.

Figure 3-31. Plot of incidence rate by dose, with fitted curve for the nested Rai and Van Ryzin model where the litter specific covariate was not used and the intra-litter correlations were estimated, for incidence of offspring loss from implantation through PND 4 in F2 offspring CRL Sprague-Dawley rats; gestational doses of F1 dams ([Ema et al., 2008](#)).

Rai and Van Ryzin Model (Version: 2.12; Date: 04/27/2015)

The form of the probability function is:
 Prob. = $[1 - \exp(-\text{Alpha} \cdot \text{Beta} \cdot \text{Dose}^\rho)] \cdot \exp(-(\text{Th1} + \text{Th2} \cdot \text{Dose}) \cdot \text{Rij})$,
 where Rij is the litter specific covariate.
 Restrict Power rho >= 1.

Benchmark Dose Computation

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all the data: 14.425287
 BMR = 5% ER
 BMD = 315.585
 BMDL at the 95% confidence level = 157.792

Parameter Estimates

Variable	Estimate	(Default) Initial parameter values
alpha	0.201085	0.201085
beta	7.58104×10^{-6}	7.58104×10^{-6}
rho	1.53267	1.53267

phi1	0.222343	0.222343
phi2	0.0213907	0.0213907
phi3	0.0759418	0.0759418
phi4	0.277171	0.277171

Log-likelihood: -610.162 AIC: 1,234.32

Goodness-of-Fit Table

Dose	Lit.-Spec. Cov.	Litter Est. Prob.	Size	Expected	Scaled Observed	Residual
0.0000	9.0000	0.182	9	1.639	3	0.7049
0.0000	10.0000	0.182	10	1.822	4	1.0303
0.0000	11.0000	0.182	11	2.004	5	1.3037
0.0000	11.0000	0.182	11	2.004	0	-0.8718
0.0000	12.0000	0.182	12	2.186	1	-0.4778
0.0000	13.0000	0.182	13	2.368	0	-0.8885
0.0000	13.0000	0.182	13	2.368	3	0.2371
0.0000	13.0000	0.182	13	2.368	3	0.2371
0.0000	13.0000	0.182	13	2.368	0	-0.8885
0.0000	14.0000	0.182	14	2.550	1	-0.5442
0.0000	14.0000	0.182	14	2.550	3	0.1579
0.0000	15.0000	0.182	15	2.732	15	4.0466
0.0000	15.0000	0.182	15	2.732	11	2.7271
0.0000	16.0000	0.182	16	2.915	4	0.3377
0.0000	16.0000	0.182	16	2.915	2	-0.2845
0.0000	16.0000	0.182	16	2.915	2	-0.2845
0.0000	16.0000	0.182	16	2.915	1	-0.5956
0.0000	16.0000	0.182	16	2.915	2	-0.2845
0.0000	16.0000	0.182	16	2.915	2	-0.2845
0.0000	17.0000	0.182	17	3.097	3	-0.0285
0.0000	17.0000	0.182	17	3.097	0	-0.9115
0.0000	17.0000	0.182	17	3.097	6	0.8546
0.0000	18.0000	0.182	18	3.279	1	-0.6365
9.7000	2.0000	0.182	2	0.365	2	2.9630
9.7000	12.0000	0.182	12	2.188	5	1.8912
9.7000	13.0000	0.182	13	2.371	3	0.4032
9.7000	13.0000	0.182	13	2.371	0	-1.5189
9.7000	13.0000	0.182	13	2.371	4	1.0439
9.7000	14.0000	0.182	14	2.553	3	0.2736
9.7000	14.0000	0.182	14	2.553	1	-0.9508
9.7000	14.0000	0.182	14	2.553	1	-0.9508
9.7000	14.0000	0.182	14	2.553	0	-1.5630
9.7000	14.0000	0.182	14	2.553	2	-0.3386
9.7000	15.0000	0.182	15	2.735	4	0.7418
9.7000	15.0000	0.182	15	2.735	4	0.7418
9.7000	15.0000	0.182	15	2.735	3	0.1552
9.7000	15.0000	0.182	15	2.735	2	-0.4314
9.7000	16.0000	0.182	16	2.918	0	-1.6437
9.7000	16.0000	0.182	16	2.918	2	-0.5170
9.7000	16.0000	0.182	16	2.918	1	-1.0803
9.7000	16.0000	0.182	16	2.918	2	-0.5170
9.7000	17.0000	0.182	17	3.100	3	-0.0543

9.7000	17.0000	0.182	17	3.100	1	-1.1386
9.7000	17.0000	0.182	17	3.100	4	0.4879
9.7000	18.0000	0.182	18	3.282	3	-0.1476
9.7000	21.0000	0.182	21	3.830	4	0.0806

100.0000	11.0000	0.189	11	2.083	3	0.5323
100.0000	11.0000	0.189	11	2.083	1	-0.6282
100.0000	12.0000	0.189	12	2.272	0	-1.2357
100.0000	13.0000	0.189	13	2.461	0	-1.2604
100.0000	14.0000	0.189	14	2.651	2	-0.3149
100.0000	14.0000	0.189	14	2.651	3	0.1691
100.0000	14.0000	0.189	14	2.651	5	1.1369
100.0000	14.0000	0.189	14	2.651	2	-0.3149
100.0000	14.0000	0.189	14	2.651	6	1.6208
100.0000	14.0000	0.189	14	2.651	1	-0.7988
100.0000	14.0000	0.189	14	2.651	2	-0.3149
100.0000	15.0000	0.189	15	2.840	1	-0.8442
100.0000	15.0000	0.189	15	2.840	2	-0.3854
100.0000	15.0000	0.189	15	2.840	0	-1.3031
100.0000	15.0000	0.189	15	2.840	3	0.0734
100.0000	16.0000	0.189	16	3.029	4	0.4235
100.0000	16.0000	0.189	16	3.029	2	-0.4491
100.0000	17.0000	0.189	17	3.219	3	-0.0910
100.0000	17.0000	0.189	17	3.219	7	1.5729
100.0000	19.0000	0.189	19	3.597	10	2.4370

995.0000	7.0000	0.393	7	2.751	7	2.0149
995.0000	10.0000	0.393	10	3.930	2	-0.6684
995.0000	11.0000	0.393	11	4.323	3	-0.4205
995.0000	12.0000	0.393	12	4.716	0	-1.3852
995.0000	12.0000	0.393	12	4.716	6	0.3772
995.0000	13.0000	0.393	13	5.109	9	1.0623
995.0000	14.0000	0.393	14	5.502	4	-0.3831
995.0000	14.0000	0.393	14	5.502	0	-1.4032
995.0000	14.0000	0.393	14	5.502	2	-0.8932
995.0000	14.0000	0.393	14	5.502	10	1.1472
995.0000	15.0000	0.393	15	5.895	8	0.5037
995.0000	15.0000	0.393	15	5.895	3	-0.6928
995.0000	15.0000	0.393	15	5.895	9	0.7430
995.0000	15.0000	0.393	15	5.895	11	1.2216
995.0000	16.0000	0.393	16	6.288	15	1.9636
995.0000	16.0000	0.393	16	6.288	4	-0.5157
995.0000	16.0000	0.393	16	6.288	2	-0.9664
995.0000	17.0000	0.393	17	6.681	6	-0.1451
995.0000	17.0000	0.393	17	6.681	1	-1.2101
995.0000	17.0000	0.393	17	6.681	5	-0.3581
995.0000	20.0000	0.393	20	7.860	6	-0.3402

Observed Chi-square = 102.1763 Bootstrap Iterations per run = 10,000
p-value = 0.1416

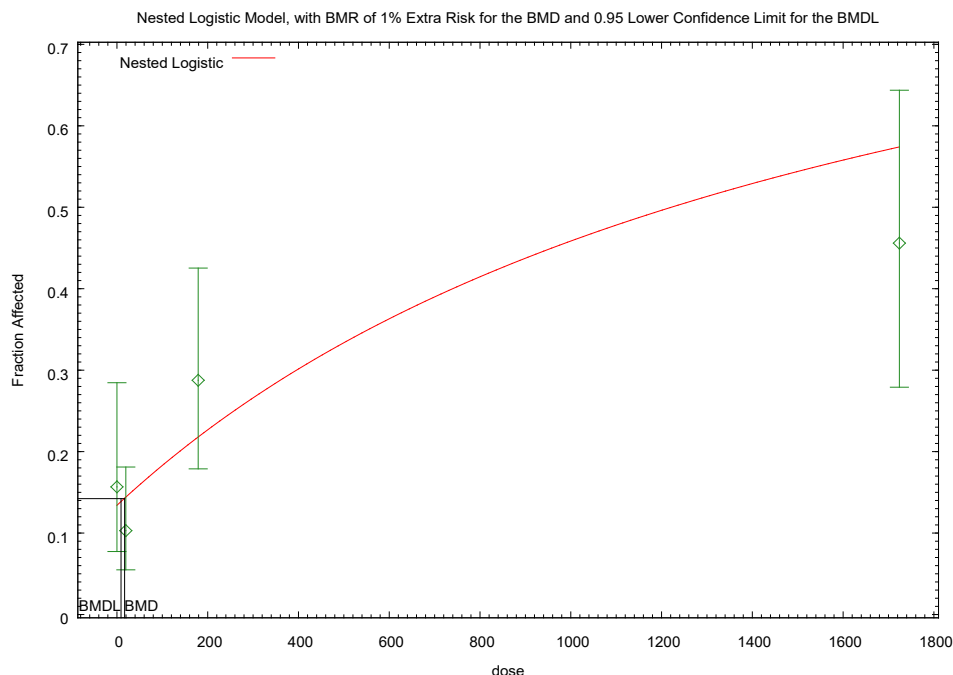
Table 3-20. Summary of BMD modeling results for offspring loss from PND 4 through PND 21 in F2 offspring CRL Sprague-Dawley rats; lactational doses of F1 dams ([Ema et al., 2008](#)); BMR = 1% ER and 5% ER

Model ^a	Goodness of Fit		BMD _{1Pct} (mg/kg-d)	BMDL _{1Pct} (mg/kg-d)	BMD _{5Pct} (mg/kg-d)	BMDL _{5Pct} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Litter-specific covariate = implantation size; intra-litter correlations estimated							Of the models that provided an adequate fit, a valid BMDL estimate and BMD/BMDL <5, the Nested Logistic model (litter-specific covariate not used; intra-litter correlations estimated) was selected based on lowest AIC (BMDLs differed by <3).
Nested Logistic	0.4417	561.04	20.4	10.1841	106.295	53.0644	
NCTR	0.4114	561.816	25.079	12.5395	127.994	63.997	
Rai and Van Ryzin	0.4056	564.38	25.8561	1.00024	131.96	5.9492	
Litter-specific covariate = implantation size; intra-litter correlations assumed to be zero							
Nested Logistic	0.0000	643.52	36.1762	22.5296	188.497	117.391	
NCTR	0.0000	650.146	33.8744	16.9372	172.883	86.4414	
Rai and Van Ryzin	0.0000	660.111	35.975	17.9875	183.603	91.8017	
Litter-specific covariate not used; intra-litter correlations estimated							
Nested Logistic	0.3944	559.472	16.9114	9.03491	88.1172	47.0766	
NCTR ^b Rai and Van Ryzin	0.4051	560.38	25.8566	12.9283	131.963	65.9814	
Litter-specific covariate not used; intra-litter correlations assumed to be zero							
Nested Logistic	0.0000	654.556	26.3666	18.3313	137.384	95.5159	
NCTR ^b Rai and Van Ryzin	0.0000	656.111	35.975	17.9875	183.603	91.8017	

^aBecause the individual animal data were available, the BMDS nested models were fitted, with the selected model in bold. For the selected model, the proportion of litters with scaled residuals above 2 in absolute value for doses 0, 19.6, 179, and 1,724 mg/kg-d were 2/22, 0/22, 2/20, and 0/20, respectively.

^bWith the litter-specific covariate not used, the NCTR and Rai and van Ryzin models yielded identical results.

Data from [Ema et al. \(2008\)](#)



BMR = 1% ER; dose shown in mg/kg-day.

Figure 3-32. Plot of incidence rate by dose, with fitted curve for the nested logistic model where the litter specific covariate was not used and the intra-litter correlations were estimated, for incidence of offspring loss from PND 4 through PND 21 in F2 offspring CRL Sprague-Dawley rats; lactational doses of F1 dams ([Ema et al., 2008](#)).

Nested Logistic Model (Version: 2.20; Date: 04/27/2015)

The form of the probability function is:

$$\text{Prob.} = \alpha + \theta_1 \cdot R_{ij} + [1 - \alpha - \theta_1 \cdot R_{ij}] / [1 + \exp(-\beta - \theta_2 \cdot R_{ij} - \rho \cdot \log(\text{Dose}))],$$

where R_{ij} is the litter specific covariate.

Restrict Power $\rho \geq 1$.

Benchmark Dose Computation

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all the data: 14.654762

BMR = 1% ER

BMD = 16.9114

BMDL at the 95% confidence level = 9.03491

Parameter Estimates

Variable	Estimate	(Default) Initial Parameter Values
alpha	0.133513	0.133513
beta	-7.42311	-7.42311
rho	1	1

phi1	0.229222	0.229222
phi2	0.152985	0.152985
phi3	0.247495	0.247495
phi4	0.586386	0.586386

Log-likelihood: -273.736 AIC: 559.472

Goodness-of-Fit Table

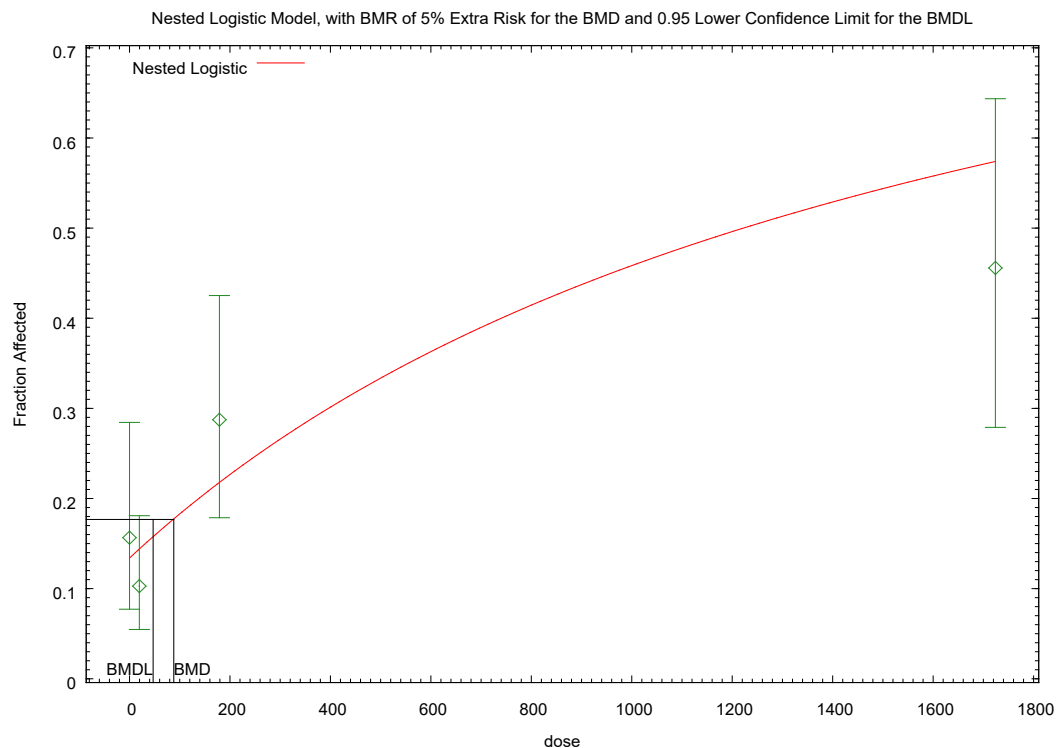
Dose	Lit.-Spec.		Litter		Scaled	
	Dose	Cov.	Est. Prob.	Size	Expected	Observed Residual
0.0000	9.0000	0.134	6	0.801	0	-0.6563
0.0000	10.0000	0.134	6	0.801	1	0.1630
0.0000	11.0000	0.134	8	1.068	0	-0.6880
0.0000	11.0000	0.134	6	0.801	0	-0.6563
0.0000	12.0000	0.134	8	1.068	1	-0.0439
0.0000	13.0000	0.134	8	1.068	6	3.1766
0.0000	13.0000	0.134	8	1.068	0	-0.6880
0.0000	13.0000	0.134	8	1.068	3	1.2443
0.0000	13.0000	0.134	8	1.068	0	-0.6880
0.0000	14.0000	0.134	8	1.068	1	-0.0439
0.0000	14.0000	0.134	8	1.068	0	-0.6880
0.0000	15.0000	0.134	4	0.534	0	-0.6043
0.0000	16.0000	0.134	8	1.068	1	-0.0439
0.0000	16.0000	0.134	8	1.068	1	-0.0439
0.0000	16.0000	0.134	8	1.068	0	-0.6880
0.0000	16.0000	0.134	8	1.068	2	0.6002
0.0000	16.0000	0.134	8	1.068	1	-0.0439
0.0000	16.0000	0.134	8	1.068	4	1.8884
0.0000	17.0000	0.134	8	1.068	0	-0.6880
0.0000	17.0000	0.134	8	1.068	0	-0.6880
0.0000	17.0000	0.134	8	1.068	5	2.5325
0.0000	18.0000	0.134	8	1.068	0	-0.6880
19.6000	12.0000	0.144	7	1.005	2	0.7747
19.6000	13.0000	0.144	8	1.148	1	-0.1039
19.6000	13.0000	0.144	8	1.148	0	-0.8046
19.6000	13.0000	0.144	8	1.148	3	1.2975
19.6000	14.0000	0.144	8	1.148	2	0.5968
19.6000	14.0000	0.144	8	1.148	0	-0.8046
19.6000	14.0000	0.144	8	1.148	0	-0.8046
19.6000	14.0000	0.144	8	1.148	0	-0.8046
19.6000	14.0000	0.144	8	1.148	0	-0.8046
19.6000	15.0000	0.144	8	1.148	1	-0.1039
19.6000	15.0000	0.144	8	1.148	3	1.2975
19.6000	15.0000	0.144	8	1.148	0	-0.8046
19.6000	15.0000	0.144	8	1.148	1	-0.1039
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	17.0000	0.144	8	1.148	1	-0.1039
19.6000	17.0000	0.144	8	1.148	0	-0.8046
19.6000	17.0000	0.144	8	1.148	3	1.2975

19.6000	18.0000	0.144	8	1.148	1	-0.1039
19.6000	21.0000	0.144	8	1.148	0	-0.8046

179.0000	11.0000	0.217	8	1.738	4	1.1735
179.0000	11.0000	0.217	8	1.738	2	0.1361
179.0000	12.0000	0.217	8	1.738	2	0.1361
179.0000	13.0000	0.217	8	1.738	0	-0.9013
179.0000	14.0000	0.217	8	1.738	2	0.1361
179.0000	14.0000	0.217	8	1.738	5	1.6922
179.0000	14.0000	0.217	8	1.738	3	0.6548
179.0000	14.0000	0.217	8	1.738	1	-0.3826
179.0000	14.0000	0.217	8	1.738	4	1.1735
179.0000	14.0000	0.217	8	1.738	1	-0.3826
179.0000	14.0000	0.217	8	1.738	6	2.2109
179.0000	15.0000	0.217	8	1.738	0	-0.9013
179.0000	15.0000	0.217	8	1.738	0	-0.9013
179.0000	15.0000	0.217	8	1.738	1	-0.3826
179.0000	15.0000	0.217	8	1.738	6	2.2109
179.0000	16.0000	0.217	8	1.738	0	-0.9013
179.0000	16.0000	0.217	8	1.738	4	1.1735
179.0000	17.0000	0.217	8	1.738	0	-0.9013
179.0000	17.0000	0.217	8	1.738	0	-0.9013
179.0000	19.0000	0.217	8	1.738	5	1.6922

1,724.0000	10.0000	0.573	8	4.585	4	-0.1850
1,724.0000	11.0000	0.573	8	4.585	2	-0.8178
1,724.0000	12.0000	0.573	8	4.585	1	-1.1341
1,724.0000	12.0000	0.573	6	3.439	0	-1.4313
1,724.0000	13.0000	0.573	4	2.292	1	-0.7865
1,724.0000	14.0000	0.573	8	4.585	8	1.0805
1,724.0000	14.0000	0.573	8	4.585	1	-1.1341
1,724.0000	14.0000	0.573	8	4.585	0	-1.4505
1,724.0000	14.0000	0.573	4	2.292	4	1.0392
1,724.0000	15.0000	0.573	7	4.012	3	-0.3637
1,724.0000	15.0000	0.573	8	4.585	0	-1.4505
1,724.0000	15.0000	0.573	6	3.439	6	1.0662
1,724.0000	15.0000	0.573	4	2.292	4	1.0392
1,724.0000	16.0000	0.573	1	0.573	1	0.8631
1,724.0000	16.0000	0.573	8	4.585	5	0.1313
1,724.0000	16.0000	0.573	8	4.585	0	-1.4505
1,724.0000	17.0000	0.573	8	4.585	3	-0.5014
1,724.0000	17.0000	0.573	8	4.585	8	1.0805
1,724.0000	17.0000	0.573	8	4.585	3	-0.5014
1,724.0000	20.0000	0.573	8	4.585	8	1.0805

Observed Chi-square = 86.7400 Bootstrap Iterations per run = 10,000
 p-value = 0.3944



13:27 08/10 2016

BMR = 5% ER; dose shown in mg/kg-day.

Figure 3-33. Plot of incidence rate by dose, with fitted curve for the nested logistic model where the litter specific covariate was not used and the intra-litter correlations were estimated, for incidence of offspring loss from PND 4 through PND 21 in F2 offspring CRL Sprague-Dawley rats; gestational doses of F1 dams (Ema et al., 2008).

Nestod Logistic Model (Version: 2.20; Date: 04/27/2015)

The form of the probability function is:

$$\text{Prob.} = \alpha + \theta_1 * R_{ij} + [1 - \alpha - \theta_1 * R_{ij}] / [1 + \exp(-\beta - \theta_2 * R_{ij} - \rho * \log(\text{Dose}))],$$

where R_{ij} is the litter specific covariate.

Restrict Power $\rho \geq 1$.

Benchmark Dose Computation

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all the data: 14.654762

BMR = 5% ER

BMD = 88.1172

BMDL at the 95% confidence level = 47.0766

Parameter Estimates

Variable	Estimate	(Default) Initial Parameter Values
alpha	0.133513	0.133513

beta	-7.42311	-7.42311
rho	1	1
phi1	0.229222	0.229222
phi2	0.152985	0.152985
phi3	0.247495	0.247495
phi4	0.586386	0.586386

Log-likelihood: -273.736 AIC: 559.472

Goodness-of-Fit Table

Dose	Lit.-Spec.		Litter		Scaled		Residual
	Cov.	Est.	Prob.	Size	Expected	Observed	
0.0000	9.0000	0.134	6	0.801	0	-0.6563	
0.0000	10.0000	0.134	6	0.801	1	0.1630	
0.0000	11.0000	0.134	8	1.068	0	-0.6880	
0.0000	11.0000	0.134	6	0.801	0	-0.6563	
0.0000	12.0000	0.134	8	1.068	1	-0.0439	
0.0000	13.0000	0.134	8	1.068	6	3.1766	
0.0000	13.0000	0.134	8	1.068	0	-0.6880	
0.0000	13.0000	0.134	8	1.068	3	1.2443	
0.0000	13.0000	0.134	8	1.068	0	-0.6880	
0.0000	14.0000	0.134	8	1.068	1	-0.0439	
0.0000	14.0000	0.134	8	1.068	0	-0.6880	
0.0000	15.0000	0.134	4	0.534	0	-0.6043	
0.0000	16.0000	0.134	8	1.068	1	-0.0439	
0.0000	16.0000	0.134	8	1.068	1	-0.0439	
0.0000	16.0000	0.134	8	1.068	0	-0.6880	
0.0000	16.0000	0.134	8	1.068	2	0.6002	
0.0000	16.0000	0.134	8	1.068	1	-0.0439	
0.0000	16.0000	0.134	8	1.068	4	1.8884	
0.0000	17.0000	0.134	8	1.068	0	-0.6880	
0.0000	17.0000	0.134	8	1.068	0	-0.6880	
0.0000	17.0000	0.134	8	1.068	5	2.5325	
0.0000	18.0000	0.134	8	1.068	0	-0.6880	
19.6000	12.0000	0.144	7	1.005	2	0.7747	
19.6000	13.0000	0.144	8	1.148	1	-0.1039	
19.6000	13.0000	0.144	8	1.148	0	-0.8046	
19.6000	13.0000	0.144	8	1.148	3	1.2975	
19.6000	14.0000	0.144	8	1.148	2	0.5968	
19.6000	14.0000	0.144	8	1.148	0	-0.8046	
19.6000	14.0000	0.144	8	1.148	0	-0.8046	
19.6000	14.0000	0.144	8	1.148	0	-0.8046	
19.6000	14.0000	0.144	8	1.148	0	-0.8046	
19.6000	15.0000	0.144	8	1.148	1	-0.1039	
19.6000	15.0000	0.144	8	1.148	3	1.2975	
19.6000	15.0000	0.144	8	1.148	0	-0.8046	
19.6000	15.0000	0.144	8	1.148	1	-0.1039	
19.6000	16.0000	0.144	8	1.148	0	-0.8046	
19.6000	16.0000	0.144	8	1.148	0	-0.8046	
19.6000	16.0000	0.144	8	1.148	0	-0.8046	
19.6000	16.0000	0.144	8	1.148	0	-0.8046	

19.6000	17.0000	0.144	8	1.148	1	-0.1039
19.6000	17.0000	0.144	8	1.148	0	-0.8046
19.6000	17.0000	0.144	8	1.148	3	1.2975
19.6000	18.0000	0.144	8	1.148	1	-0.1039
19.6000	21.0000	0.144	8	1.148	0	-0.8046
179.0000	11.0000	0.217	8	1.738	4	1.1735
179.0000	11.0000	0.217	8	1.738	2	0.1361
179.0000	12.0000	0.217	8	1.738	2	0.1361
179.0000	13.0000	0.217	8	1.738	0	-0.9013
179.0000	14.0000	0.217	8	1.738	2	0.1361
179.0000	14.0000	0.217	8	1.738	5	1.6922
179.0000	14.0000	0.217	8	1.738	3	0.6548
179.0000	14.0000	0.217	8	1.738	1	-0.3826
179.0000	14.0000	0.217	8	1.738	4	1.1735
179.0000	14.0000	0.217	8	1.738	1	-0.3826
179.0000	14.0000	0.217	8	1.738	6	2.2109
179.0000	15.0000	0.217	8	1.738	0	-0.9013
179.0000	15.0000	0.217	8	1.738	0	-0.9013
179.0000	15.0000	0.217	8	1.738	1	-0.3826
179.0000	15.0000	0.217	8	1.738	6	2.2109
179.0000	16.0000	0.217	8	1.738	0	-0.9013
179.0000	16.0000	0.217	8	1.738	4	1.1735
179.0000	17.0000	0.217	8	1.738	0	-0.9013
179.0000	17.0000	0.217	8	1.738	0	-0.9013
179.0000	19.0000	0.217	8	1.738	5	1.6922

1,724.0000	10.0000	0.573	8	4.585	4	-0.1850
1,724.0000	11.0000	0.573	8	4.585	2	-0.8178
1,724.0000	12.0000	0.573	8	4.585	1	-1.1341
1,724.0000	12.0000	0.573	6	3.439	0	-1.4313
1,724.0000	13.0000	0.573	4	2.292	1	-0.7865
1,724.0000	14.0000	0.573	8	4.585	8	1.0805
1,724.0000	14.0000	0.573	8	4.585	1	-1.1341
1,724.0000	14.0000	0.573	8	4.585	0	-1.4505
1,724.0000	14.0000	0.573	4	2.292	4	1.0392
1,724.0000	15.0000	0.573	7	4.012	3	-0.3637
1,724.0000	15.0000	0.573	8	4.585	0	-1.4505
1,724.0000	15.0000	0.573	6	3.439	6	1.0662
1,724.0000	15.0000	0.573	4	2.292	4	1.0392
1,724.0000	16.0000	0.573	1	0.573	1	0.8631
1,724.0000	16.0000	0.573	8	4.585	5	0.1313
1,724.0000	16.0000	0.573	8	4.585	0	-1.4505
1,724.0000	17.0000	0.573	8	4.585	3	-0.5014
1,724.0000	17.0000	0.573	8	4.585	8	1.0805
1,724.0000	17.0000	0.573	8	4.585	3	-0.5014
1,724.0000	20.0000	0.573	8	4.585	8	1.0805

Observed Chi-square = 86.7400 Bootstrap Iterations per run = 10,000
p-value = 0.4003

Table 3-21. Summary of BMD modeling results for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose([Ema et al., 2008](#)); BMR = 5% RD from control mean, 10% RD from control mean, 0.5 SD change from control mean, and 1 SD change from control mean

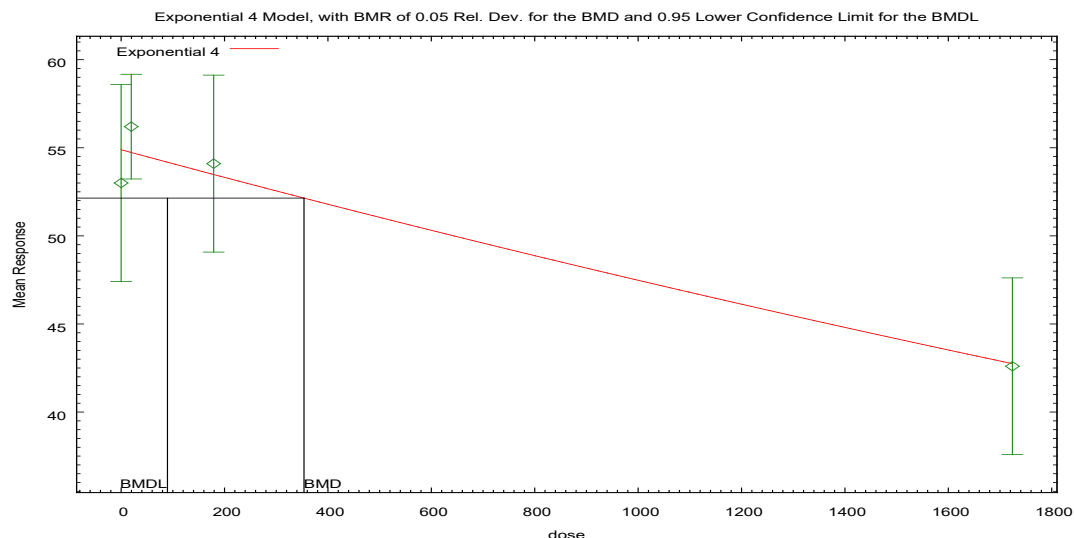
Model ^a	Goodness of fit		BMD _{5RD} (mg/kg-d)	BMDL _{5RD} (mg/kg-d)	BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2)	0.486	420.90	354	240	727	494	Of the models that provided an adequate fit, a valid BMDL estimate and BMD/BMDL <5, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M3)	0.266	422.69	651	244	1016	500	
Exponential (M4)	0.486	420.90	354	89.6	727	206	
Exponential (M5)	N/A ^b	424.68	230	94.0	258	181	
Hill	N/A ^b	424.68	230	89.2	264	error ^c	
Power	0.266	422.69	676	282	1,049	565	
Polynomial 3 ^o Polynomial 2 ^o	0.264	422.70	817	282	1,161	564	
Linear	0.497	420.85	389	280	779	560	
Model ^a	Goodness of fit		BMD _{0.5SD} (mg/kg-d)	BMDL _{0.5SD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)	
	p-value	AIC					
Exponential (M2)	0.486	420.90	634	419	1,332	879	
Exponential (M3)	0.266	422.69	937	425	1,483	891	
Exponential (M4)	0.486	420.90	634	172	1,332	468	
Exponential (M5)	N/Ab	424.68	252	176	296	189	
Hill	N/Ab	424.68	256	176	324	error ^c	
Power	0.266	422.69	969	482	1,503	965	
Polynomial 3 ^o Polynomial 2 ^o	0.264	422.70	1,091	482	1,549	964	
Linear	0.497	420.85	684	478	1,368	956	

^aConstant variance case presented (BMDS Test 2 p-value = 0.0278), selected model in bold; scaled residuals for selected model for doses 0, 19.6, 179, and 1,724 mg/kg-day were -0.92, 0.71, 0.27, and -0.06, respectively.

^bNo available degrees of freedom to calculate a goodness-of-fit value.

^cBMD or BMDL computation failed for this model.

Data from [Ema et al. \(2008\)](#)



BMR = 5% RD from control mean; dose shown in mg/kg-day.

Figure 3-34. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose ([Ema et al., 2008](#)).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 5% RD

BMD = 353.728

BMDL at the 95% confidence level = 89.5935

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	4.53195	4.51269
rho	N/A	0
a	54.8883	59.01
b	0.000145008	0.00128594
c	0	0.687535
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	53	54.89	12.6	9.64	-0.9187
19.6	22	56.2	54.73	6.7	9.64	0.714

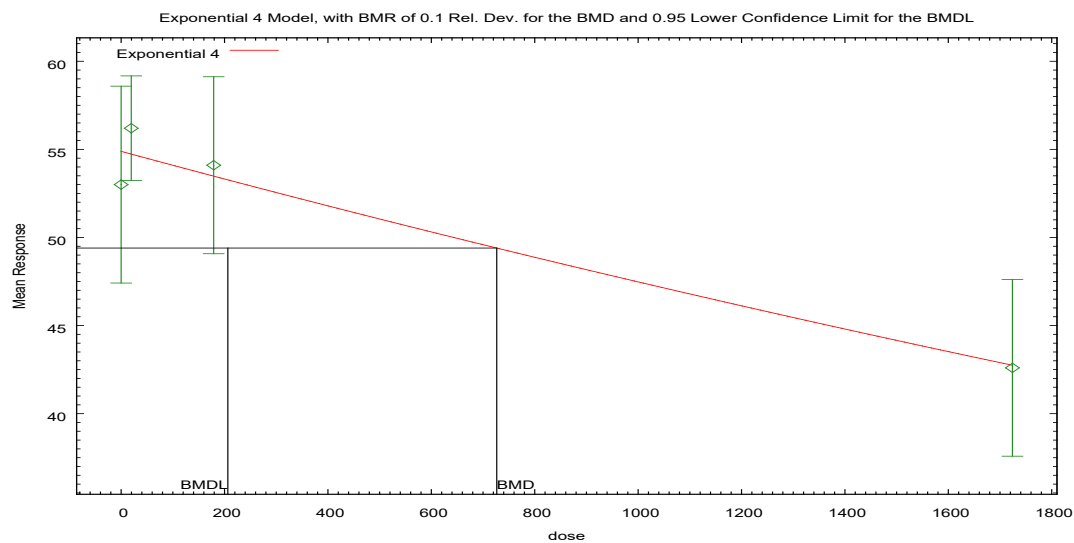
179	18	54.1	53.48	10.1	9.64	0.272
1,724	13	42.6	42.75	8.3	9.64	-0.0551

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-206.7258	5	423.4517
A2	-202.1665	8	420.333
A3	-206.7258	5	423.4517
R	-214.7267	2	433.4535
4	-207.4482	3	420.8963

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	25.12	6	0.0003244
Test 2	9.119	3	0.02775
Test 3	9.119	3	0.02775
Test 6a	1.445	2	0.4856



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure 3-35. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose ([Ema et al., 2008](#)).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 726.585

BMDL at the 95% confidence level = 206.377

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	4.53195	4.51269
rho	N/A	0
a	54.8883	59.01
b	0.000145008	0.00128594
c	0	0.687535
d	N/A	1

Table of Data and Estimated Values of Interest

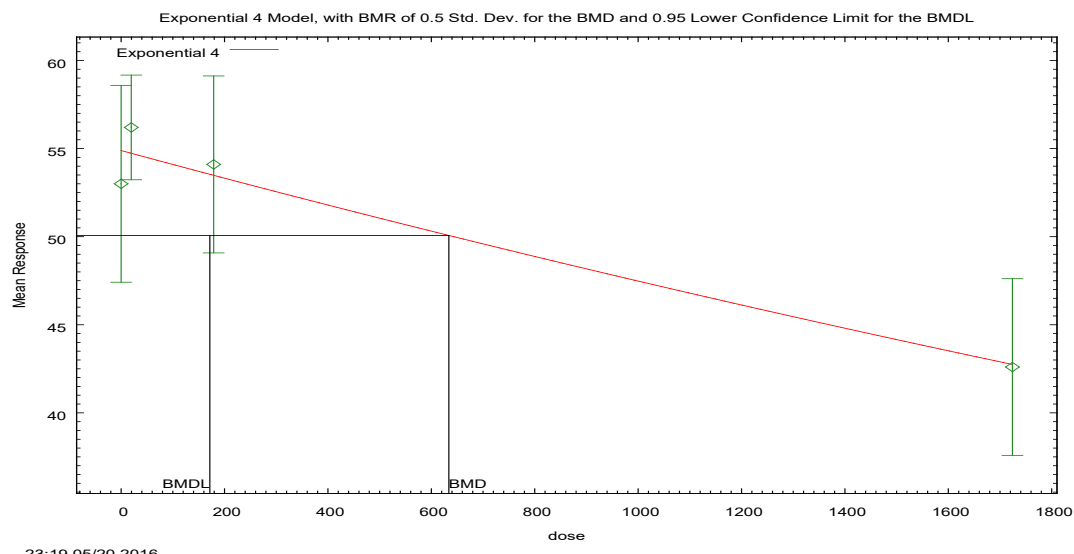
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	53	54.89	12.6	9.64	-0.9187
19.6	22	56.2	54.73	6.7	9.64	0.714
179	18	54.1	53.48	10.1	9.64	0.272
1,724	13	42.6	42.75	8.3	9.64	-0.0551

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-206.7258	5	423.4517
A2	-202.1665	8	420.333
A3	-206.7258	5	423.4517
R	-214.7267	2	433.4535
4	-207.4482	3	420.8963

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	25.12	6	0.0003244
Test 2	9.119	3	0.02775
Test 3	9.119	3	0.02775
Test 6a	1.445	2	0.4856



BMR = 0.5 SD change from control mean; dose shown in mg/kg-day.

Figure 3-36. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 50% Estimated SDs from control

BMD = 633.879

BMDL at the 95% confidence level = 171.599

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	4.53195	4.51269
rho	N/A	0
a	54.8883	59.01
b	0.000145008	0.00128594
c	0	0.687535
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
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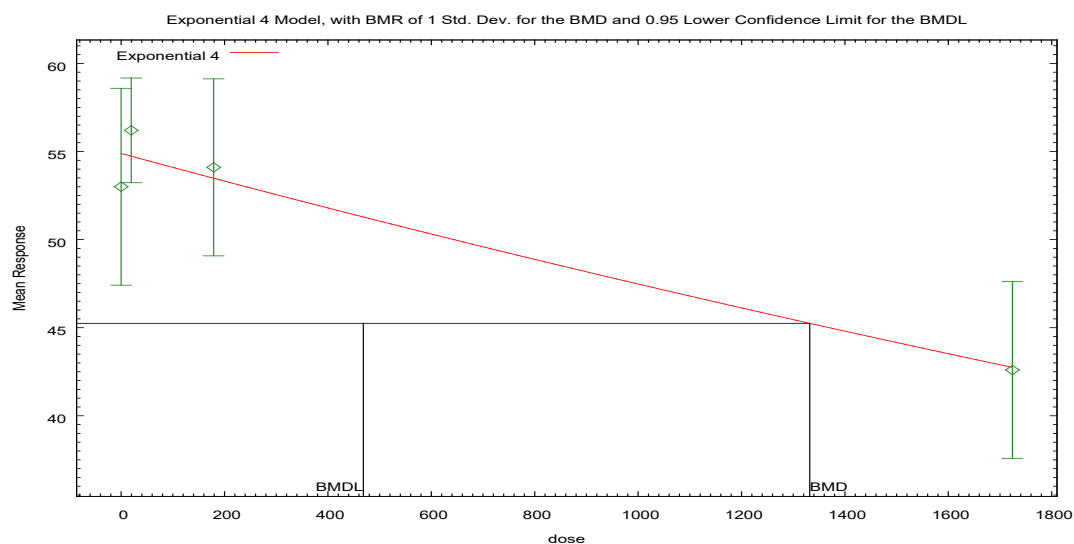
0	22	53	54.89	12.6	9.64	-0.9187
19.6	22	56.2	54.73	6.7	9.64	0.714
179	18	54.1	53.48	10.1	9.64	0.272
1,724	13	42.6	42.75	8.3	9.64	-0.0551

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-206.7258	5	423.4517
A2	-202.1665	8	420.333
A3	-206.7258	5	423.4517
R	-214.7267	2	433.4535
4	-207.4482	3	420.8963

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	25.12	6	0.0003244
Test 2	9.119	3	0.02775
Test 3	9.119	3	0.02775
Test 6a	1.445	2	0.4856



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure 3-37. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose (Ema et al., 2008).

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: $Y[\text{dose}] = a * [c - (c - 1) * \exp(-b * \text{dose})]$

A constant variance model is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 1331.98

BMDL at the 95% confidence level = 468.431

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	4.53195	4.51269
rho	N/A	0
a	54.8883	59.01
b	0.000145008	0.00128594
c	0	0.687535
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	53	54.89	12.6	9.64	-0.9187
19.6	22	56.2	54.73	6.7	9.64	0.714
179	18	54.1	53.48	10.1	9.64	0.272
1,724	13	42.6	42.75	8.3	9.64	-0.0551

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-206.7258	5	423.4517
A2	-202.1665	8	420.333
A3	-206.7258	5	423.4517
R	-214.7267	2	433.4535
4	-207.4482	3	420.8963

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	25.12	6	0.0003244
Test 2	9.119	3	0.02775
Test 3	9.119	3	0.02775
Test 6a	1.445	2	0.4856

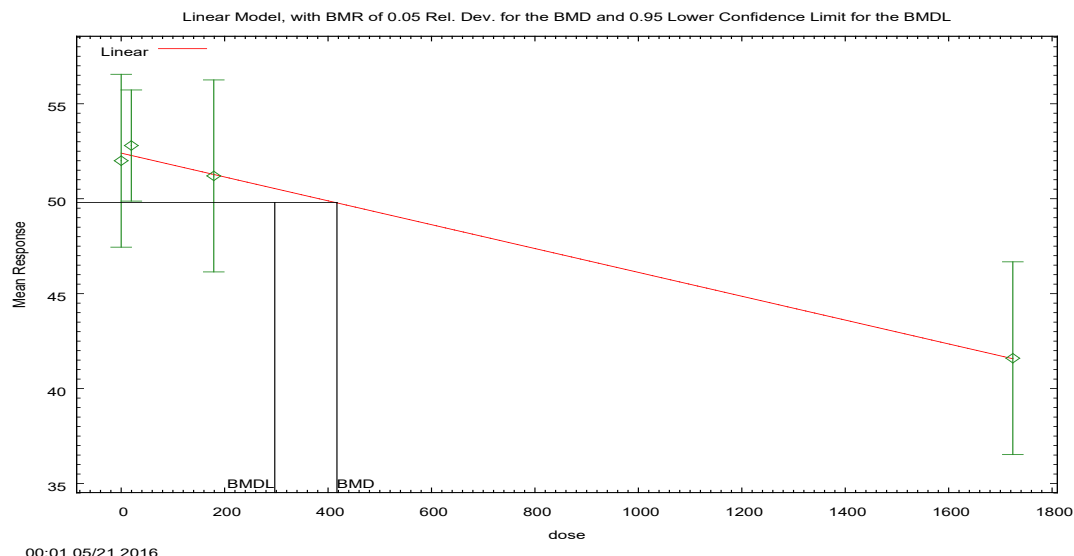
Table 3-22. Summary of BMD modeling results for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose (Ema et al., 2008); BMR = 5% RD from control mean, 10% RD from control mean, 0.5 SD change from control mean and 1 SD change from control mean

Model ^a	Goodness of fit		BMD _{5RD} (mg/kg-d)	BMDL _{5RD} (mg/kg-d)	BMD _{10RD} (mg/kg-d)	BMDL _{10RD} (mg/kg-d)	Basis for model selection	
	p-value	AIC						
Exponential (M2)	0.942	413.8640	381	257	783	528	Of the models that provided an adequate fit, a valid BMDL estimate and BMD/BMDL <5, the Linear constant variance model was selected based on lowest AIC (BMDLs differed by <3).	
Exponential (M3)	0.732	415.86	411	257	815	529		
Exponential (M4)	0.729	415.86	381	257	783	528		
Exponential (M5)	N/A ^b	417.83	201	76.5	225	179		
Hill	N/A ^b	417.83	203	67.7	235	error ^c		
Power	0.729	415.86	423	297	840	594		
Polynomial 3 ^o Polynomial 2 ^o Linear	0.942	413.8637	417	297	834	594		
Model _a	Goodness of fit		BMD _{0.5SD} (mg/kg-d)	BMDL _{0.5SD} (mg/kg-d)	BMD _{1SD} (mg/kg-d)	BMDL _{1SD} (mg/kg-d)		
	p-value	AIC						
Exponential (M2)	0.942	413.864	657	432	1378	903		
Exponential (M3)	0.732	415.86	690	432	1397	903		
Exponential (M4)	0.729	415.86	657	432	1378	903		
Exponential (M5)	N/Ab	417.83	219	140	256	188		
Hill	N/Ab	417.83	226	133	291	error _c		
Power	0.729	415.86	712	489	1,416	978		
Polynomial 3 ^o Polynomial 2 ^o Linear	0.942	413.8637	706	489	1,412	978		

^aConstant variance case presented (BMDS Test 2 p-value = 0.133), selected model in bold; scaled residuals for selected model for doses 0, 19.6, 179, and 1,724 mg/kg-day were -0.22, 0.26, -0.05, and 0, respectively.

^bNo available degrees of freedom to calculate a goodness-of-fit value.

^cBMD or BMDL computation failed for this model.



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 BMR = 5% RD from control mean; dose shown in mg/kg-day.

Figure 3-38. Plot of mean response by dose with fitted curve for Linear model with constant variance for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose (Ema et al., 2008).

Polynomial Model (Version: 2.20; Date: 10/22/2014)

The form of the response function is: $Y[\text{dose}] = \text{beta}_0 + \text{beta}_1 * \text{dose}$
 A constant variance model is fit

Benchmark Dose Computation

BMR = 5% RD
 BMD = 417.145
 BMDL at the 95% confidence level = 296.948

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	78.7776	83.0228
rho	N/A	0
beta_0	52.4269	52.4168
beta_1	-0.00628402	-0.00627654

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	52	52.4	10	8.88	-0.22
19.6	22	52.8	52.3	6.6	8.88	0.262
179	20	51.2	51.3	10.8	8.88	-0.0514

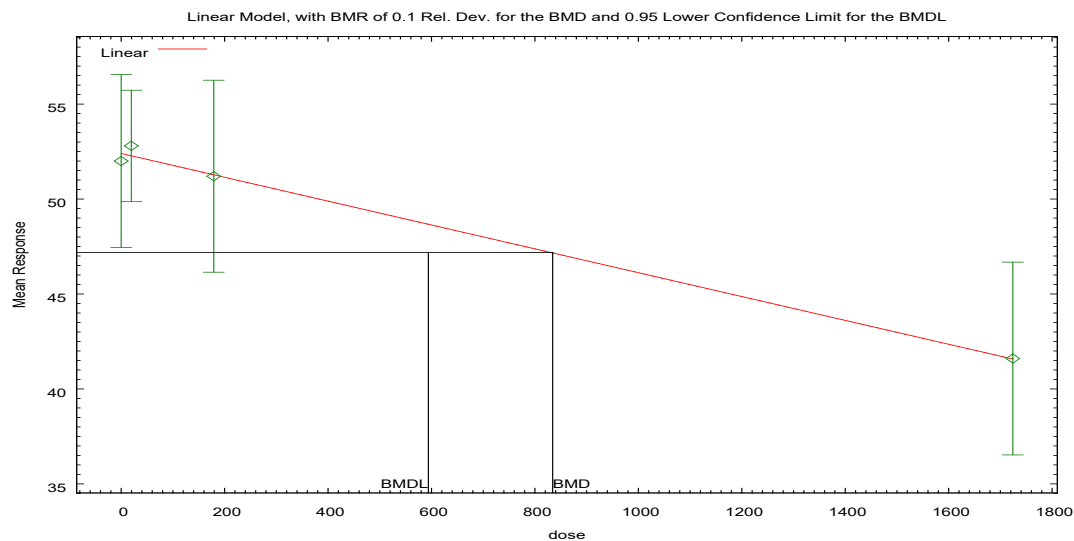
1,724	13	41.6	41.6	8.4	8.88	0.00274
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Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-203.871816	5	417.743631
A2	-201.070527	8	418.141053
A3	-203.871816	5	417.743631
fitted	-203.931869	3	413.863738
R	-210.813685	2	425.627371

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	19.4863	6	0.003416
Test 2	5.60258	3	0.1326
Test 3	5.60258	3	0.1326
Test 4	0.120106	2	0.9417



00:07 05/21 2016
 BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure 3-39. Plot of mean response by dose with fitted curve for Linear model with constant variance for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose (Ema et al., 2008).

Polynomial Model (Version: 2.20; Date: 10/22/2014)

The form of the response function is: $Y[\text{dose}] = \text{beta}_0 + \text{beta}_1 * \text{dose}$
 A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 834.289

BMDL at the 95% confidence level = 593.896

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	78.7776	83.0228
rho	N/A	0
beta_0	52.4269	52.4168
beta_1	-0.00628402	-0.00627654

Table of Data and Estimated Values of Interest

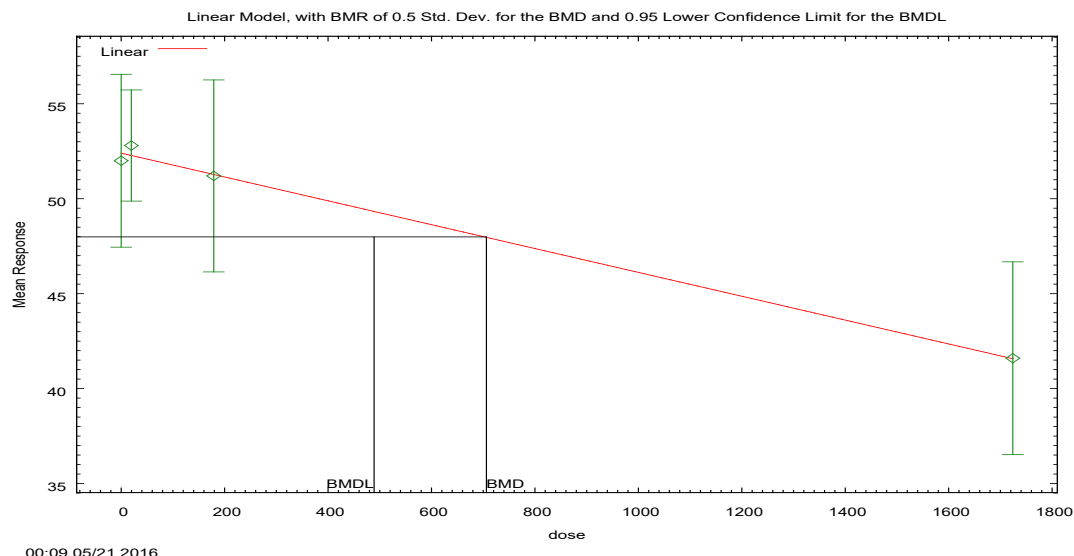
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	52	52.4	10	8.88	-0.22
19.6	22	52.8	52.3	6.6	8.88	0.262
179	20	51.2	51.3	10.8	8.88	-0.0514
1,724	13	41.6	41.6	8.4	8.88	0.00274

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-203.871816	5	417.743631
A2	-201.070527	8	418.141053
A3	-203.871816	5	417.743631
fitted	-203.931869	3	413.863738
R	-210.813685	2	425.627371

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	19.4863	6	0.003416
Test 2	5.60258	3	0.1326
Test 3	5.60258	3	0.1326
Test 4	0.120106	2	0.9417



BMR = 0.5 SD change from control mean; dose shown in mg/kg-day.

Figure 3-40. Plot of mean response by dose with fitted curve for Linear model with constant variance for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose (Ema et al., 2008).

Polynomial Model (Version: 2.20; Date: 10/22/2014)

The form of the response function is: $Y[\text{dose}] = \text{beta}_0 + \text{beta}_1 * \text{dose}$

A constant variance model is fit

Benchmark Dose Computation

BMR = 50% Estimated SDs from the control mean

BMD = 706.21

BMDL at the 95% confidence level = 488.985

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	78.7776	83.0228
rho	N/A	0
beta_0	52.4269	52.4168
beta_1	-0.00628402	-0.00627654

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	52	52.4	10	8.88	-0.22
19.6	22	52.8	52.3	6.6	8.88	0.262
179	20	51.2	51.3	10.8	8.88	-0.0514

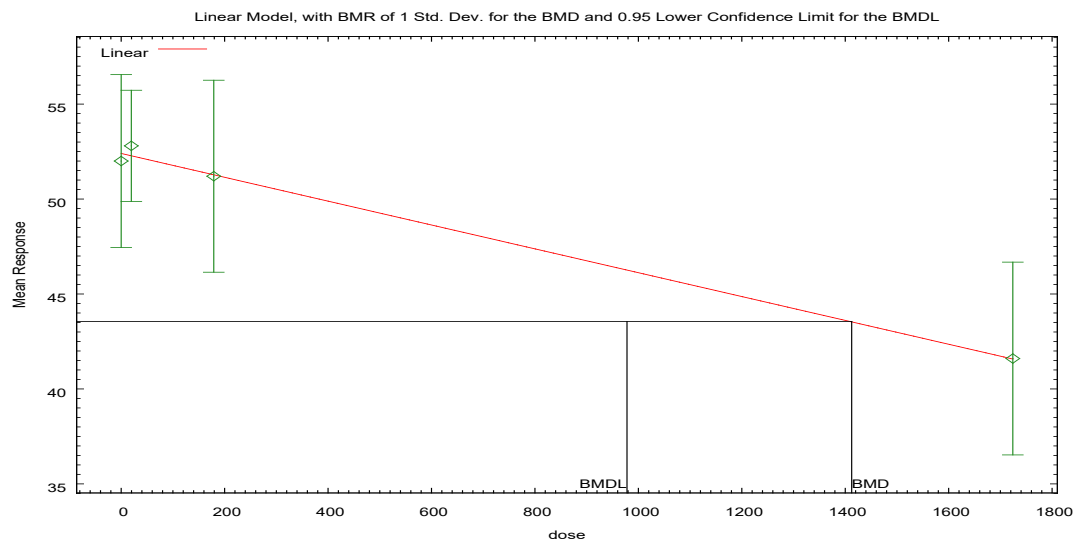
1,724	13	41.6	41.6	8.4	8.88	0.00274
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Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-203.871816	5	417.743631
A2	-201.070527	8	418.141053
A3	-203.871816	5	417.743631
fitted	-203.931869	3	413.863738
R	-210.813685	2	425.627371

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	19.4863	6	0.003416
Test 2	5.60258	3	0.1326
Test 3	5.60258	3	0.1326
Test 4	0.120106	2	0.9417



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure 3-41. Plot of mean response by dose with fitted curve for Linear model with constant variance for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose (Ema et al., 2008).

Polynomial Model (Version: 2.20; Date: 10/22/2014)

The form of the response function is: $Y[\text{dose}] = \text{beta}_0 + \text{beta}_1 * \text{dose}$

A constant variance model is fit

Benchmark Dose Computation

BMR = 1 Estimated SDs from the control mean

BMD = 1412.42

BMDL at the 95% confidence level = 977.97

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	78.7776	83.0228
rho	N/A	0
beta_0	52.4269	52.4168
beta_1	-0.00628402	-0.00627654

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	52	52.4	10	8.88	-0.22
19.6	22	52.8	52.3	6.6	8.88	0.262
179	20	51.2	51.3	10.8	8.88	-0.0514
1,724	13	41.6	41.6	8.4	8.88	0.00274

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-203.871816	5	417.743631
A2	-201.070527	8	418.141053
A3	-203.871816	5	417.743631
fitted	-203.931869	3	413.863738
R	-210.813685	2	425.627371

Tests of Interest

Test	-2*log (likelihood ratio)	Test df	p-value
Test 1	19.4863	6	0.003416
Test 2	5.60258	3	0.1326
Test 3	5.60258	3	0.1326
Test 4	0.120106	2	0.9417

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