United States
Environmental Protection Agency

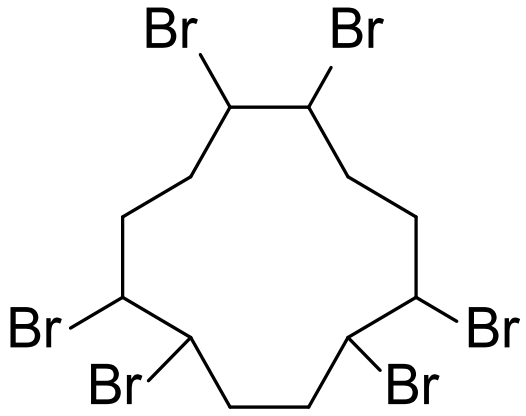
Office of Chemical Safety and
Pollution Prevention

# Final Risk Evaluation for Cyclic Aliphatic Bromides Cluster (HBCD)

## Supplemental Information File:

## Supplemental Information on Human Health Hazard

**CASRN: 25637-99-4**
**CASRN: 3194-55-6**
**CASRN: 3194-57-8**




***September 2020***

# TABLE OF CONTENTS

# 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 (, 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**

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td colspan="2">Studies in infants</td></tr>
<tr><td rowspan="3">Eggesbø et al. (2011) (Norway, 2003−2006)<br>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)<br>Exposure measures: Breast milk, collected at a median of 33 d after delivery (samples pooled over 8 consecutive mornings)<br>Total HBCD detected in 67.9% of samples<br>LOQ = 0.2 ng/g lipid<br>Median 0.54 (range: 0.1−31) ng/g lipid<br>Effect measures: TSH (whole blood spots) measured in infants 3 d after delivery (linked data beginning in February 2004); immunoassay (clinical lab)<br>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.<br><br>Data Quality:<br>High (1.4)</td><td>Association between HBCD level in breast milk with neonatal TSH levels:<br><table><tr><th>Exposure category (ng/g lipid) (N)</th><th>Adjusted beta for ln TSH (95% CI)[b]</th><th>Adjusted odds ratio for TSH ≥80th percentile (95% CI)[c]</th></tr><tr><td>0.10 (62)</td><td>(Referent)</td><td>(Referent)</td></tr><tr><td>0.13−0.52 (31)</td><td>−0.01 (−0.21, 0.20)</td><td>1.3 (0.3, 4.5)</td></tr><tr><td>0.53−0.79 (33)</td><td>0.02 (−0.18, 0.22)</td><td>1.4 (0.3, 6.1)</td></tr><tr><td>0.80−1.24 (33)</td><td>0.12 (−0.08, 0.33)</td><td>1.6 (0.4, 6.1)</td></tr><tr><td>1.29−31.2 (34)</td><td>0.03 (−0.17, 0.23)</td><td>1.3 (0.3, 5.8)</td></tr><tr><td>Per interquartile range increase:</td><td>−0.00 (−0.02, 0.02)</td><td>1.0 (0.8, 1.1)</td></tr></table></td></tr>
<tr><td>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, oxychlordane, and sum of all PCB congeners.<br><br>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.</td></tr>
</table>

| Reference and study design | Results |
|---|---|
| Roze et al. (2009) (the Netherlands, COMPARE cohort, 2001−2002)<br>**Population:** Birth cohort, 90 singleton, term births, 62 of 69 (90%) mother-child pairs randomly selected from the cohort for HBCD measures in serum<br>**Exposure measures:** Prenatal exposure, maternal serum at 35th week of pregnancy<br>1,2,5,6,9,10-HBCD (HBCD) detected in all samples<br>LOD 0.8 pg/g serum<br>Median 0.8 (range: 0.3−7.5) ng/g lipids<br>**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)<br>**Analysis:** Pearson correlation (for normally distributed variables) or Spearman's rank correlation (for non-normally distributed variables)<br><br>**Data Quality**: [a]<br>Medium (1.8) | Results for correlations between HBCD and cord blood thyroid hormone levels were not shown, but were stated to be not statistically significant. |
| Kim and Oh (2014) (South Korea, 2009−2010)<br>**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.<br>**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<br>LOQ 0.036 ng/g lipid (% less than detection limit not reported)<br>Total HBCD: Mean 8.55 ng/g lipid, range from less than method detection limit to 166 ng/g lipid<br>**Effect measures:** Congenital hypothyroidism (not defined)<br>**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.<br><br>**Data Quality**: [a]<br>Medium (1.9) | Congenital hypothyroidism Healthy controls<br>Mothers, mean HBCD level (SD)<br>α-HBCD 0.494 (1.52) 2.57 (1.48)*<br>β-HBCD 0.27 (0.933) 0.461 (1.08)<br>γ-HBCD 2.72 (1.42) 8.86 (2.81)<br>Infants, mean HBCD level (SD)<br>α-HBCD 2.42 (3.33) 1.84 (2.5)<br>β-HBCD 0.578 (1.71) 0.462 (0.768)<br>γ-HBCD 5.16 (2.42) 14.05 (2.87) |
| *Studies in adolescents* | |
| Kiciński et al. (2012) (Belgium, 2008−2011)<br>**Population:** 515 adolescents (13−17 yrs old) from two industrial sites and randomly selected from the | Thyroid hormone results (estimated from Figure 4 of Kiciński et al. (2012):<br>Beta (95% CI)[d] |

| Reference and study design | Results | |
|---|---|---|
| general population; participation rates 22−34% in the three groups, sample size varied by test<br>**Exposure measures:** Serum samples, HBCD >75% were less than the LOQ (LOQ = 30 ng/L); Median <30 (range: <LOQ−234) ng/L<br>**Effect measures:**<br>Thyroid hormones:<br>Free T3, free T4, TSH (immunoassay not described)<br>**Analysis:** Regression models (linear or negative binomial depending on outcome); HBCD dichotomized<br><br>**Data Quality**: [a]<br>Medium (1.9) | Free T3 (pg/mL)<br>FreeT4 (mg/dL)<br>TSH (%) | 0.08 (−0.08, 2.3)<br>−0.02 (−0.03, 0.09)<br>0.0 (−4, 13) |
| | 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. | |
| *Studies in adult men* | | |
| Johnson et al. (2013) (United States, 2002−2003)<br>**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<br>**Exposure measures:** HBCD exposure from vacuum bag dust; three main stereoisomers of HBCD presented together<br>HBCD detected in 97% of samples; LOD not reported; median 246 ng/g dust (90th percentile 1,103 ng/g dust)<br>**Effect measures:** Non-fasting blood sample immunoassay details in (Meeker et al., 2008)<br>TSH<br>free T4<br>free T3<br>**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<br><br>**Data Quality**: [a]<br>High (1.6) | Adjustment for age and BMI produced similar results to the bivariate results (data not reported).<br><br>No statistically significant changes in thyroid hormones (result not shown). | |

*$p$ = 0.004; unadjusted for age and sex.
[a]Based on OPPT data evaluation criteria
[b]0.0 = no association.
[c]1.0 = no association.
[d]Beta 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 | | | | |
|---|---|---|---|---|---|
| *Serum thyroid hormones* | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning through necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/lactation<br><br>Thyroid hormones were measured by radioimmunoassay in adults only<br><br>**Data Quality**: [d]<br>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) | |
| | % of control[a] | – | −2% | | −10% | | −11% | |
| van der Ven et al. (2009)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating<br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>Thyroid hormones (total T3/T4) were measured by radioimmunoassay in adults only<br><br>**Data Quality**: [d]<br>High (1.2) | **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 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| WIL Research (2001)<br>Rats, Crl:CD(SD)IGS BR<br>Gavage<br>90-d exposure starting on ~PNW 7 followed by a 28-d recovery period<br><br>Recovery data not shown<br><br>Thyroid hormones (total T3/T4) measured by electro-chemiluminescence immunoassay in adults only<br><br>**Data Quality**: [d]<br>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. | | **0** | **100** | **300** | **1,000** | | | |
| | **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% | | | |
| van der Ven et al. (2006)<br>Rats, Wistar<br>Gavage<br>28-d exposure starting on PNW 11<br><br>Thyroid hormones (total T3/T4) were measured by radioimmunoassay<br><br>**Data Quality**: [d]<br>High (1.3) | **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)<br>Rats, Crj:CD(SD)IGS<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11<br>Thyroid hormones were measured by electrochemi-luminescence immunoassay in males only<br><br>**Data Quality**: [d]<br>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)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning until necropsy<br>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 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Incidence | 0/24 | | 1/24 | | 5/24* | | 13/24* |
| **Data Quality**: [d] High (1.0) | **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 |
| | **Female, F1** (n = 24) | | | | | | | |
| | Incidence | 0/24 | | 0/24 | | 0/24 | | 0/24 |
| | **Thyroid gland histopathology** | | | | | | | |
| | Treatment-related histopathological thyroid changes were not observed in weanling F1 and F2 animals. | | | | | | | |
| WIL Research (2001)<br>Rats, Crl:CD(SD)IGS BR<br>Gavage<br>90-d exposure starting on ~PNW 7 followed by a 28-d recovery period<br><br>Recovery data not shown<br><br>**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)<br>Rats, Wistar<br>Gavage<br>28-d exposure in adults starting on PNW 11<br><br>**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)<br>Rats, Sprague-Dawley<br>Gavage<br>28-d exposure starting on ~PNW 6 followed by a 14-d recovery period<br><br>**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)<br>Rats, Crj:CD(SD)IGS<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11<br><br>**Data Quality**: [d]<br>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)<br>Mice, BALB/c<br>Females only<br>Diet<br>28-d exposure starting on PND 26<br><br>**Data Quality**: [d]<br>High (1.3) | **Doses** (mg/kg-d) | | | |
| | **0** | | **199** | |
| | **Female** (n = 6–8) | | | |
| | **Colloid area** (µm$^2$) | | | |
| | Mean (SD) | 1,718 (403) | | 1,270 (452) |
| | % of control[a] | – | | –26% |
| | **Follicle area** (µm$^2$) | | | |
| | 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% |
| *Thyroid weight* | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning through necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/lactation<br><br>Thyroid weight measured in adults only<br><br>**Data Quality**: [d]<br>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 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| van der Ven et al. (2009)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating<br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data Quality**: [d]<br>High (1.2) | | **0** | **0.1** | **0.3** | **1** | **3** | **10** | **30** | **100** |
| | **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)<br>Rats, Crl:CD(SD)IGS BR<br>Gavage<br>90-d exposure starting on ~PNW 7 followed by a 28-d recovery period<br><br>Recovery data not shown<br><br>**Data Quality**: [d]<br>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)<br>Rats, Wistar<br>Gavage<br>28-d exposure starting on PNW 11<br><br>**Data Quality**: [d]<br>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)<br>Rats, Crj:CD(SD)IGS<br>Diet<br><br>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% | |
| | **Male, F1, PNW 11** (n = 10) | | | | | | | | |

| Reference and study design | Results | | | | |
|---|---|---|---|---|---|
| exposure period through PNW 11<br><br>**Data Quality**: [d] High (1.2) | 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.
[a]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[b]Not measured; only control and high-dose values reported for endocrine parameters in the F0 animals.
[c]Time-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.
[d]Based on OPPT data evaluation criteria

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

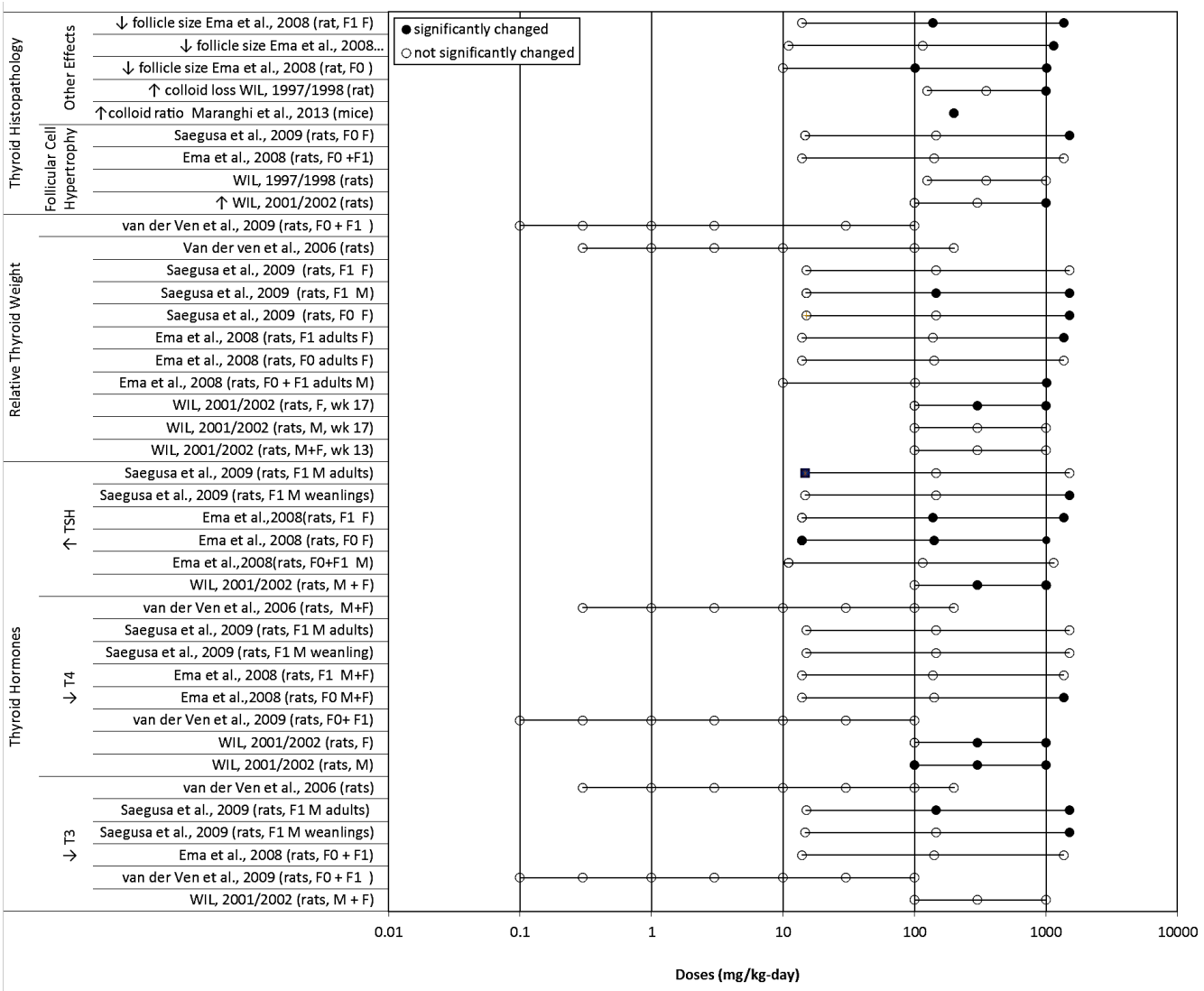



**Figure 1-1. Exposure response array of thyroid effects following oral exposure. All studies scored a High in data quality evaluation.**

### 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). Pentoxyresorufin-O-depentylase 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 TRα 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).

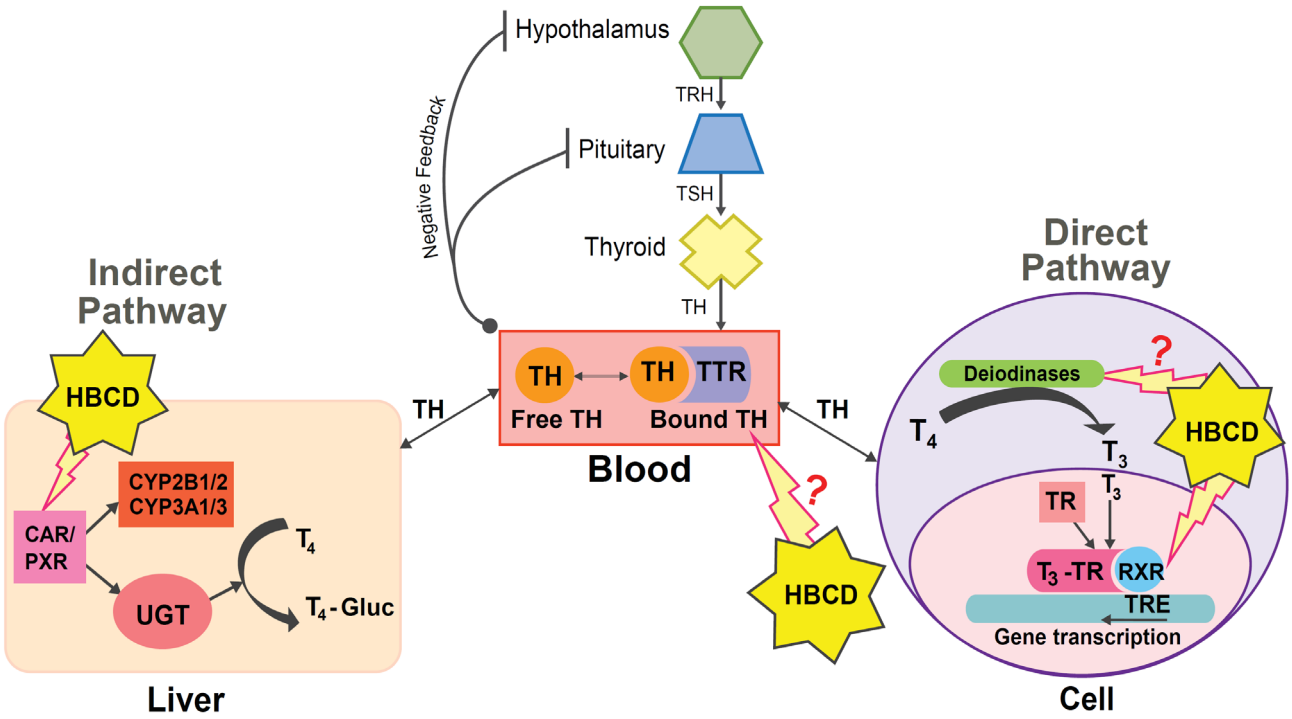



**Figure 1-2. Hypothesized MOAs for thyroid effects of HBCD (adapted from Miller et al. (2009))**

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 = constituative antrostane receptor/pregnane X receptor; Gluc = glucuronide; RXR = retinoid X receptor; $T_4$ = Thyroxine; $T_3$ = triiodothyronine; TH = thyroid hormone; TR = thyroid receptor; TRE = thyroid hormone response element; TRH = thyrotropin-releasing hormone; TSH = thyroid stimulating hormone; TTR = transthretin; UGT = uridine diphosphate glucuronyltransferase;

#### 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 ($IC_{50}$ > 10 µM), 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 (Emea, 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 $B_6$ (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 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Liver weight* | | | | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning until necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/lactation<br><br>**Data Quality**: [e]<br>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)<br>Rats, Wistar<br>Diet<br>One generation<br><br>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** |
| | **Absolute liver weight** (g) | | | | | | | | |
| | **Male, F1, PNW 11** (n = 4–5) | | | | | | | | |
| | 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% |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| estrous cycles (females: 14 d) prior to mating<br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data Quality**: [e]<br>High (1.2) | **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% |
| WIL Research (2001)<br>Rats, Crl:CD(SD)IGS BR<br>Gavage<br>90-d exposure starting on ~PNW 7 followed by a 28-d recovery period<br><br>Recovery data not shown<br><br>**Data Quality**: [e]<br>High (1.0) | **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% |
| van der Ven et al. (2006)<br>Rats, Wistar<br>Gavage<br>28-d exposure starting on PNW 11<br><br>**Data Quality**: [e]<br>High (1.3) | **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% |
| WIL Research (1997)<br>Rats, Sprague-Dawley<br>Gavage<br>28-d exposure starting on ~PNW 6 followed by a 14-d recovery period<br><br>Recovery data not shown<br><br>**Data Quality**: [e]<br>High (1.3) | **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% |
| | **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% |

| Reference and study design | Results | | | |
|---|---|---|---|---|
| Saegusa et al. (2009)<br>Rats, Crj:CD(SD)IGS<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11<br><br>**Data Quality**: [e]<br>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)<br>Mice, C57BL/6<br>Males only<br>Gavage<br>Animals dosed once weekly<br>15-week exposure starting on PNW 6<br>Dose groups split between standard and high-fat diets<br><br>**Data Quality**: [e]<br>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)<br>Mice, BALB/c<br>Females only<br>Diet<br>28-d exposure starting on PND 26<br><br>**Data Quality**: [e]<br>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% | |
| *Liver histopathology* | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation | **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** |

| Reference and study design | Results | | | | |
|---|---|---|---|---|---|
| F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning until necropsy<br>F1/F2: continuous maternal exposure throughout gestation/ lactation<br><br>**Data Quality**: [e]<br>High (1.3) | **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. | | | | |
| WIL Research (2001)<br>Rats, Crl:CD(SD)IGS BR<br>Gavage<br>90-d exposure starting on ~PNW 7 followed by a 28-d recovery period<br><br>Recovery data not shown<br><br>**Data Quality**: [e]<br>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. | | | | |
| WIL Research (1997)<br>Rats, Sprague-Dawley<br>Gavage<br>28-d exposure starting on ~PNW 6 followed by a 14-d recovery period<br><br>Recovery data not shown<br><br>**Data Quality**: [e]<br>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. | | | | |
| Saegusa et al. (2009)<br>Crj:CD(SD)IGS, rat<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11 | **Doses** (mg/kg-d)[c] | **0** | **15** | **146** | **1,505** |
| | **Hepatocellular vacuolar degeneration** | | | | |
| | **Male, F1, PND 20** (n = 10) | | | | |
| | Incidence | 0/10 | 0/10 | 0/10 | 6/10* |
| | **Female, F1, PND 20** (n = 10) | | | | |
| | Incidence | 0/10 | 0/10 | 0/10 | 6/10* |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Data Quality**: [e]<br>High (1.2) | | | | | | | | |
| Yanagisawa et al. (2014)<br>Mice, C57BL/6<br>Males only<br>Gavage<br>Animals dosed once weekly<br>15-wk exposure starting on PNW 6<br><br>Dose groups split between standard and high-fat diets<br><br>**Data Quality**: [e]<br>Unacceptable (4)* | **Doses** (mg/kg-wk)<br>**0** **0.00175** **0.035** **0.7**<br>**Hepatocyte ballooning**<br>The study authors observed development of hepatocyte ballooning following oral high-dose exposure in male mice fed a high-fat diet.<br>**Microvesicular fatty changes**<br>The study authors observed development of severe microvesicular fatty changes following oral high-dose exposure in male mice fed a high-fat diet.<br>Treatment-related effects were not observed in mice fed a standard diet. | | | | | | | |
| Maranghi et al. (2013)<br>BALB/c, mice<br>Females only<br>Diet<br>28-d exposure starting on PND 26<br><br>**Data Quality**: [e]<br>High (1.3) | **Doses** (mg/kg-d)<br>**0** **199**<br>**Periportal lymphatic filtration**<br>Incidence 0/10 6/8*<br>**Tissue congestion**<br>Incidence 0/10 6/8*<br>**Vacuolation in hepatocytes**<br>Incidence 0/10 5/8* | | | | | | | |
| *Liver chemistry* | | | | | | | | |
| van der Ven et al. (2009)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating<br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data Quality**: [e]<br>High (1.2) | **Doses** (mg/kg-d) | | | | | | | |
| | **0** | **0.1** | **0.3** | **1** | **3** | **10** | **30** | **100** |
| | **ALT** (U/L) | | | | | | | |
| | **Male** (n = 4–5) | | | | | | | |
| | Mean (SD) | 37.3 (1.8) | 33.6 (4.7) | 43.6 (7.8) | 43.1 (4.2) | 43.3 (4.4) | 40.3 (6.8) | 38.2 (4.7) | 37.2 (2.6) |
| | % of control[b] | – | −10% | 17% | 16% | 16% | 8% | 2.4% | 0% |
| | **Female** (n = 5) | | | | | | | |
| | Mean (SD) | 34.7 (3.3) | 37.5 (6.5) | 39.7 (12.6) | 37.3 (4.8) | 33.5 (6.2) | 30.7 (6.2) | 33.9 (10.4) | 34.0 (4.6) |
| | % of control[b] | – | 8% | 14% | 7% | −3% | −12% | −2% | −2% |
| | **ALP** (U/L) | | | | | | | |
| | **Male** (n = 4–5) | | | | | | | |
| | Mean (SD) | 3.22 (2.24) | 4.40 (2.31) | 3.28 (1.76) | 4.80 (2.79) | 3.38 (1.90) | 3.20 (0.85) | 4.60 (2.43) | 3.76 (1.90) |
| | % of control[b] | – | 37% | 2% | 49% | 5% | −1% | 43% | 17% |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Female** (n = 5)** | | | | | | | |
| | Mean (SD) | 3.78 (1.97) | 2.70 (2.37) | 3.82 (3.23) | 2.64 (0.95) | 1.14 (0.53) | 3.82 (1.64) | 2.66 (1.55) | 1.28 (0.59) |
| | % of control[b] | – | −29% | 1% | −30% | −70% | 1% | −30% | −66% |
| WIL Research (2001)<br>Rats, Crl:CD(SD)IGS BR<br>Gavage<br>90-d exposure starting on ~PNW 7 followed by a 28-d recovery period<br><br>Recovery data not shown<br><br>**Data Quality**: [c]<br>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 | | | |
| van der Ven et al. (2006)<br>Rats, Wistar<br>Gavage<br>28-d exposure starting on PNW 11 | **Doses** (mg/kg-d) | **0** | **0.3** | **1** | **3** | **10** | **30** | **100** | **200** |
| | **ALT** (U/L) | | | | | | | | |
| | **Male** (n = 3−5) | | | | | | | | |
| | Mean (SD) | 44.5 (5.9) | 40.9 (4.1) | 44.3 (10.3) | 38.2 (3.6) | 45.0 (14.3) | 42.7 (11.0) | 40.6 (8.1) | 39.2 (10.9) |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | % of control[b] | – | −8% | 0% | −14% | 1% | −4% | −9% | −12% |
| | **Female** (n = 3−5) | | | | | | | | |
| | Mean (SD) | 43.4 (4.6) | 44.7 (6.5) | 39.8 (4.5) | 40.5 (6.7) | 34.6 (6.6) | 38.2 (5.0) | 36.0 (5.2) | 42.5 (7.5) |
| | % of control[b] | – | 3% | −8% | −7% | −20% | −12% | −17% | −2% |
| **Data Quality**: [e] High (1.3) | **ALP** (U/L) | | | | | | | | |
| | **Male** (n = 3−5) | | | | | | | | |
| | Mean (SD) | 7.34 (5.59) | 5.30 (3.66) | 3.68 (1.82) | 7.43 (7.43) | 4.88 (5.75) | 5.10 (2.54) | 2.74 (1.61) | 3.48 (1.95) |
| | % of control[b] | – | −28% | −50% | 1% | −34% | −31% | −63% | −53% |
| | **Female** (n = 3−5)** | | | | | | | | |
| | Mean (SD) | 4.66 (2.91) | 3.10 (2.76) | 4.74 (2.50) | 3.72 (2.14) | 2.30 (1.21) | 2.36 (0.33) | 2.73 (1.55) | 2.42 (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<br><br>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% | | |
| | **Female** (n = 6) | | | | | | | | |
| | Mean (SD) | 75 (13.0) | | 63 (11.5) | 61 (9.6) | | 62 (9.9) | | |
| | % of control[b] | – | | −16% | −19% | | −17% | | |
| **Data Quality**: [e] | **GGT** (U/L) | | | | | | | | |

| Reference and study design | Results | | | |
|---|---|---|---|---|
| High (1.3) | **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)<br>Mice, C57BL/6<br>Males only<br>Gavage<br>Animals dosed once weekly<br>15-week exposure starting on PNW 6<br><br>Dose groups split between standard and high-fat diets<br><br>**Data Quality**: [c]<br>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.
[a]F1 and F2 offspring presented as mean maternal gestational and lactational F0 and F1 doses, respectively.
[b]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[c]TWAs 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.
[d]Based 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
[e]Based on OPPT data evaluation criteria

SE = standard error

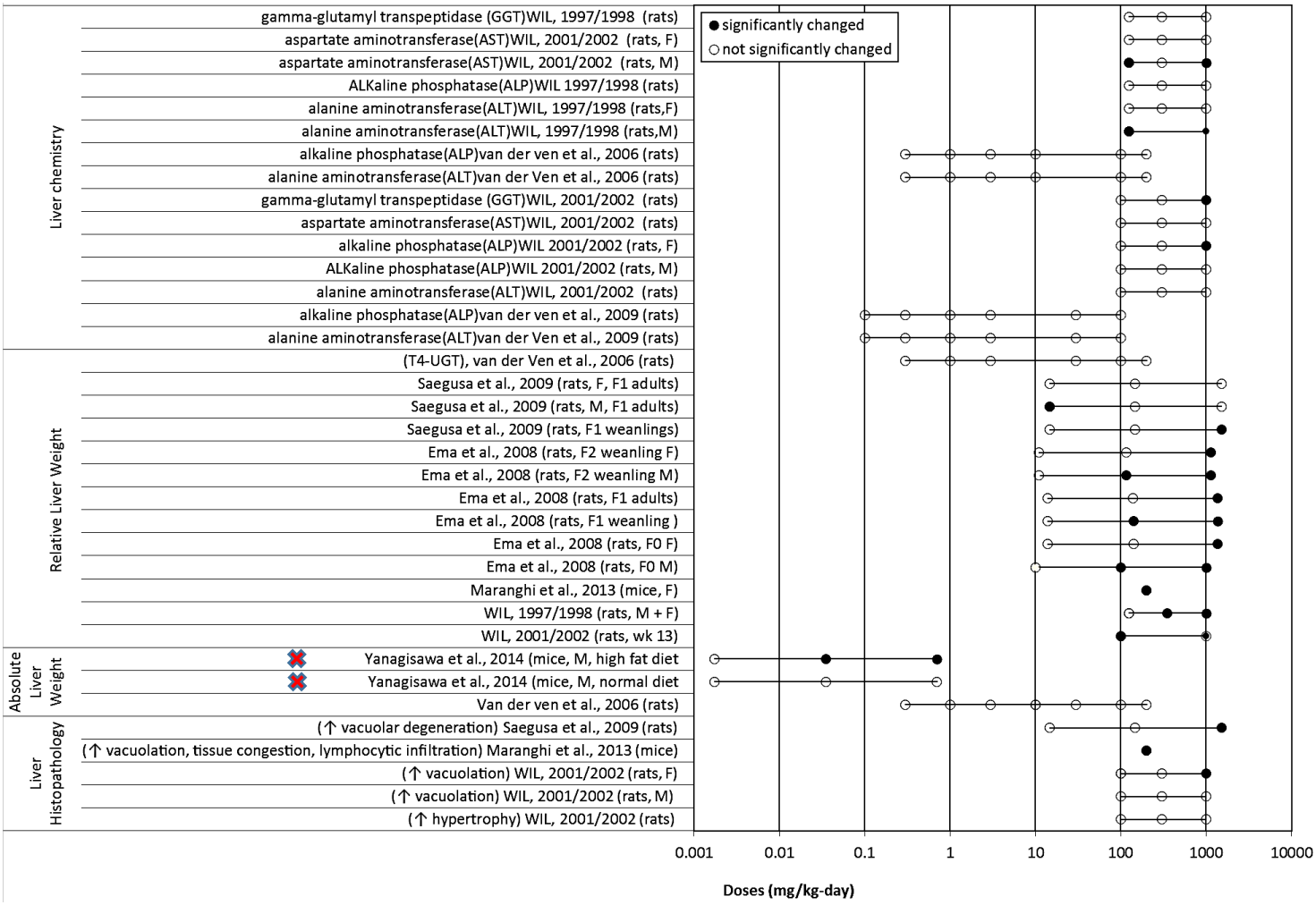



**Figure 1-3. Exposure response array of liver effects following oral exposure. All studies scored a High in data quality evaluation except for Yanagisawa et al. (2014), which scored Unacceptable. The study is included only for reference (indicated in the chart by X).**

### 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 (PPARg) 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/17β-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 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Fertility and pregnancy outcomes* | | | | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning through necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/lactation<br><br>**Data Quality**: [d]<br>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)<br>Rats, Wistar<br>Diet<br>One generation | **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 |

| Reference and study design | Results | | | |
|---|---|---|---|---|
| F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating<br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data Quality**: [d]<br>High (1.0) | | | | |
| | **Other pregnancy outcomes** | | | |
| | No significant dose-response trend in other outcomes (*e.g.*, number of implantation sites, gestation duration, litter size) | | | |
| Saegusa et al. (2009)<br>Crj:CD(SD)IGS, rat<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11<br><br>**Data Quality**: [d]<br>High (1.2) | **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) | | | |
| *Hormonal measures* | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning through necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/lactation<br><br>**Data Quality**: [d]<br>High (1.0) | **Doses** (mg/kg-d) | | | |
| | **Female, F0** | **0** | **14** | **141** | **1,363** |
| | **Female, F1** | **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. | | | |
| Maranghi et al. (2013)<br>Mice, BALB/c<br>Females only<br>Diet | **Doses** (mg/kg-d) | | | |
| | **0** | | **199** | |
| | **Testosterone** (ng/mL) | | | |
| | **Female** (n = 10) | | | |
| | Mean (SD) | 0.07 (0.02) | | 0.11* (0.07) |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 28-d exposure starting on PND 26<br><br>**Data Quality**: [d]<br>High (1.3) | % 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. | | | | | | | |
| *Reproductive differentiation and development* | | | | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning through necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/lactation<br><br>**Data Quality**: [d]<br>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)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating<br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data Quality**: [d]<br>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)<br>Crj:CD(SD)IGS, rat<br>Diet | **Doses** (mg/kg-d)[c] | **0** | **15** | **146** | **1,505** | | | | |
| | **Time to vaginal opening** (d) | | | | | | | | |
| | **Female F1** (n = 12–14) | | | | | | | | |

| 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<br><br>**Data Quality**: [d]<br>High (1.3) | 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 | | | | |
| *Reproductive organ weights* | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning through necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/lactation<br><br>**Data Quality**: [d]<br>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) | | | | |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **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) | | | | | | | |
| | 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)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating<br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data Quality**: [d]<br>High (1.2) | **Doses** (mg/kg-d) | | | | | | | |
| | | **0** | **0.1** | **0.3** | **1** | **3** | **10** | **30** | **100** |
| | **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) |
| | % of control[a] | – | 21% | 11% | 9% | 24% | 8% | 17% | 7% |
| | **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% |
| WIL Research (2001)<br>Rats, Crl:CD(SD)IGS BR<br>Gavage<br>90 d exposure starting on ~PNW 7 followed by a 28-d recovery period<br><br>Recovery data not shown<br><br>**Data Quality**: [d]<br>High (1.0) | **Doses** (mg/kg-d) | | | | | | | |
| | | **0** | **100** | **300** | **1,000** | | | |
| | **Absolute ovary with oviduct weight** (g) | | | | | | | |
| | **Female** (n = 10) | | | | | | | |
| | Mean (SD) | 0.14 (0.03) | 0.13 (0.03) | 0.13 (0.03) | 0.15 (0.02) | | | |
| | % of control[a] | – | −10% | −9% | 3% | | | |
| | **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) | | | |
| | % of control[a] | – | −8% | −12% | 2% | | | |
| | **Absolute uterus with cervix weight** (g) | | | | | | | |
| | **Female** (n = 10) | | | | | | | |
| | Mean (SD) | 0.81 (0.25) | 0.64 (0.16) | 0.67 (0.14) | 0.62 (0.17) | | | |
| | % of control[a] | – | −21% | −17% | −23% | | | |
| | **Relative uterus with cervix weight** (g/100 g BW) | | | | | | | |
| | **Female** (n = 10) | | | | | | | |

| Reference and study design | Results | | | |
|---|---|---|---|---|
| | Mean (SD) | 0.29 (0.07) | 0.23 (0.05) | 0.22 (0.04) | 0.22 (0.07) |
| | % of control[a] | – | −20% | −21% | −23% |
| WIL Research (1997)<br>Rats, Sprague-Dawley<br>Gavage<br>28-d exposure starting on ~PNW 6 followed by a 14-d recovery period<br><br>Recovery data not shown<br><br>**Data Quality**: [d]<br>High (1.3) | **Doses** (mg/kg-d) | | | | |
| | | **0** | **125** | **350** | **1,000** |
| | **Relative ovary with oviduct weight** (g/100 g BW) | | | | |
| | **Female** (n = 6) | | | | |
| | 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)<br>Rats, Crj:CD(SD)IGS<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11<br><br>**Data Quality**: [d]<br>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)<br>Mice, BALB/c<br>Females only<br>Diet<br>28-d exposure starting on PND 26<br><br>**Data Quality**: [d]<br>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.
[a]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[b]Exact number of animals examined per dose group was unclear in the published paper.

[c]TWAs 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.
[d]F1 and F2 offspring doses presented as maternal F0 and F1 mean gestational and lactational doses, respectively.

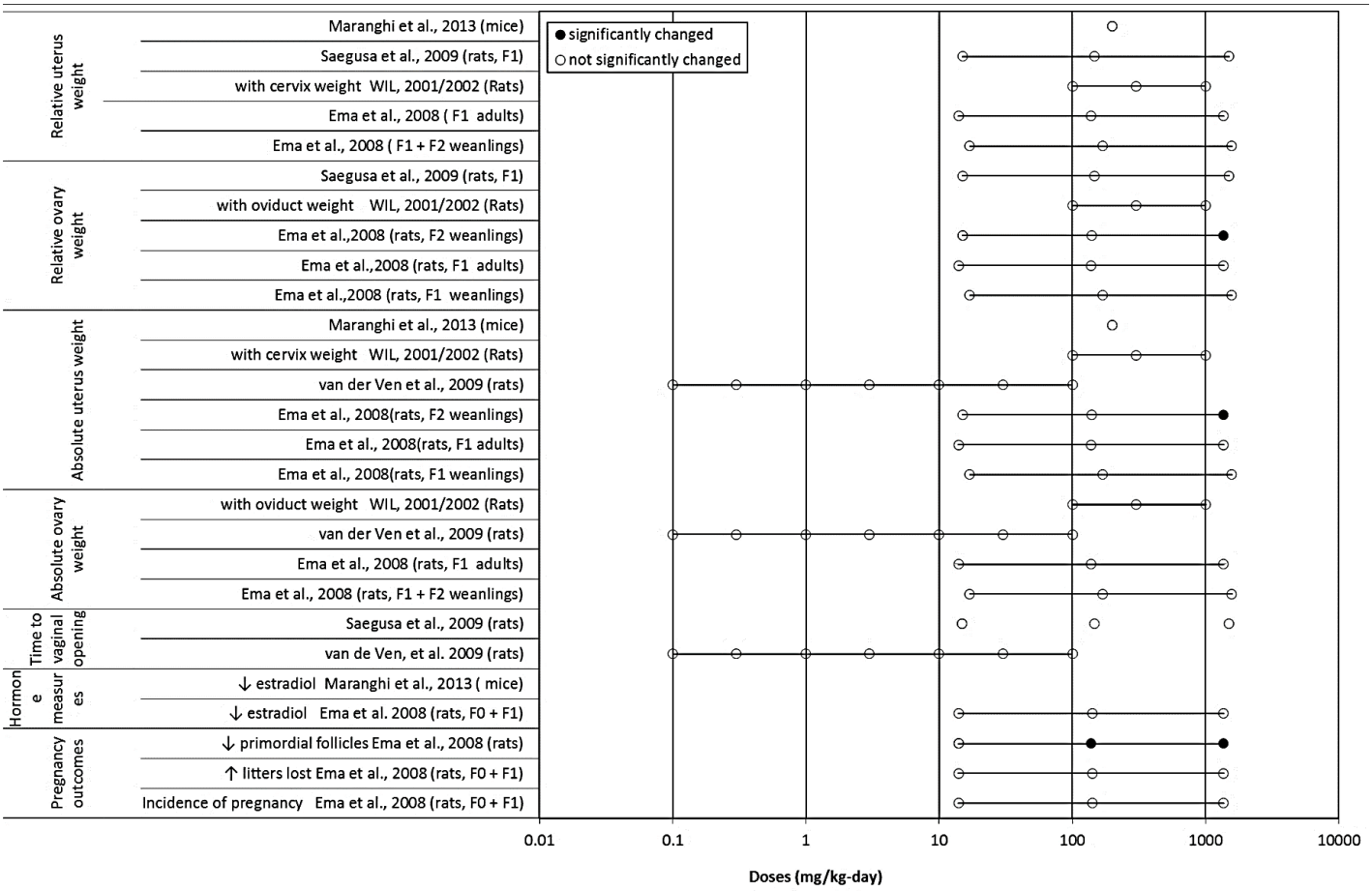



**Figure 1-4. Exposure response array of female reproductive system effects following oral exposure. All studies scored a High in data quality evaluation.**

### 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 HSD17β (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 **Error! Reference source not found.**. 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 |
|---|---|
| Meijer et al. (2012) (the Netherlands, COMPARE cohort, 2001−2002)<br>**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<br>**Exposure measures:** Prenatal exposure, maternal serum at 35th week of pregnancy<br>1,2,5,6,9,10-HBCD (HBCD) detected in 43 of 44 samples<br>LOD 0.8 pg/g serum; LOQ = 9 pg/g serum<br>Median 0.7 (range: <LOD−7.4) ng/g lipid | Spearman correlation between HBCD in maternal serum and free testosterone: r = −0.31 ($0.05 < p$-value $< 0.10$).<br><br>Correlations with other hormones noted as not statistically significant, but effect estimates were not reported.<br><br>No significant correlations between prenatal exposure to HBCD and testes volume or penile length were found (data not shown). |

| Reference and study design | Results |
|---|---|
| **Effect measures:** Reproductive hormones (serum, collected at 3 mo) (immunoassay details in immunoassay details in Laven et al., 2004)<br>• testosterone<br>• SHBG<br>• FSH<br>• LH<br>• estradiol<br>• inhibin B<br>Testes volume, measured by ultrasound (ages 3 and 18 mo); penile length (ages 3 and 18 mo)<br>**Analysis:** Spearman correlation<br><br>**Data quality:**[a]<br>Medium (1.9) | |
| Johnson et al. (2013) (USA, 2002−2003)<br>**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<br>**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)<br>**Effect measures:** Non-fasting blood sample (immunoassay details in immunoassay details in Meeker et al., 2008)<br>testosterone<br>Sex hormone binding globulin (SHBG)<br>Follicle stimulating hormone (FSH)<br>Luteinizing hormone (LH)<br>estradiol<br>inhibin B<br>prolactin<br>**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<br><br>**Data quality:**[a]<br>High (1.6) | Spearman r (*p*-value)<br>Free androgen index (testosterone/SHBG) 0.46 (*p* = 0.004)<br>SHBG −0.35[a] (*p* = 0.03)<br>Multivariate models adjusted for age and BMI reportedly produced similar results to the bivariate results (data not reported for HBCD).<br><br>Results for other hormones not shown.<br><br>Note that HBCD was not strongly correlated with other flame retardants measured (Spearman correlation coefficients ranging from −0.20 to 0.27, all *p*-values > 0.10) |

[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 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Reproductive differentiation and development* | | | | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning through necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/lactation<br><br>**Data quality:**[e]<br>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)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating<br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data quality:**[e]<br>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)<br>Rats, Crj:CD(SD)IGS<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11<br><br>**Data quality:**[e] | **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) | | | | | | | | |
| *Spermatogenic measures* | | | | | | | | |
| van der Ven et al. (2009)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating<br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data quality:**[e]<br>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)<br>Rats, Wistar<br>Gavage<br>28-d exposure starting on PNW 11<br><br>**Data quality:**[e]<br>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% |
| *Reproductive organ weights* | | | | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning through necropsy<br>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:**[e] 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<br><br>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<br><br>**Data quality:**[e]<br>High (1.2) | **Absolute prostate weight** (g) | | | | | | | | |
| | **Male, F1, PNW 11** (n = 4–5)** | | | | | | | | |
| | Mean (SD) | 0.66 (0.18) | 0.73 (0.21) | 0.57 (0.15) | 0.73 (0.21) | 0.57 (0.12) | 0.58 (0.07) | 0.67 (0.09) | 0.42 (0.13) |
| | % change[b] | – | 11% | −14% | 11% | −14% | −12% | 2% | −36% |
| | **Absolute seminiferous vesicle weight** (g) | | | | | | | | |
| | **Male, F1, PNW 11** (n = 4–5) | | | | | | | | |
| | Mean (SD) | 1.00 (0.40) | 1.07 (0.22) | 1.32 (0.23) | 1.14 (0.29) | 1.21 (0.09) | 1.07 (0.29) | 1.21 (0.25) | 1.09 (0.27) |
| | % change[b] | – | 7% | 32% | 14% | 21% | 7% | 21% | 9% |
| WIL Research (2001)<br>Rats, Crl:CD(SD)IGS BR<br>Gavage<br>90 d exposure starting on ~PNW 7 followed by a 28-d recovery period<br><br>Recovery data not shown<br><br>**Data quality:**[e]<br>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 (SD) | 0.18 (0.03) | | 0.19 (0.03) | | 0.21 (0.04) | | 0.26 (0.05) | |
| | % change[b] | – | | 3% | | 17% | | 42% | |
| | **Relative testis weight** (left) (g/100 g BW) | | | | | | | | |
| | **Male** (n = 9–10) | | | | | | | | |
| | Mean (SD) | 0.30 (0.08) | | 0.31 (0.04) | | 0.31 (0.04) | | 0.32 (0.04) | |
| | % change[b] | – | | 4% | | 2% | | 7% | |
| | **Relative testis weight (right)** (g/100 g BW) | | | | | | | | |
| | **Male** (n = 9–10) | | | | | | | | |
| | Mean (SD) | 0.31 (0.07) | | 0.31 (0.04) | | 0.31 (0.04) | | 0.32 (0.05) | |
| | % change[b] | – | | 0% | | 1% | | 6% | |
| | **Relative cauda epididymis weight** (left) (g/100 g BW) | | | | | | | | |
| | **Male** (n = 9–10) | | | | | | | | |
| | Mean (SD) | 0.05 (0.01) | | 0.06 (0.01) | | 0.06 (0.01) | | 0.06 (0.01) | |
| | % change[b] | – | | 9% | | 6% | | 15% | |
| | **Relative cauda epididymis weight** (right) (g/100 g BW) | | | | | | | | |
| | **Male** (n = 9–10) | | | | | | | | |
| | Mean (SD) | 0.05 (0.01) | | 0.06 (0.01) | | 0.06 (0.01) | | 0.06 (0.01) | |
| | % change[b] | – | | 6% | | 4% | | 17% | |
| | **Relative epididymis weight (left)** (g/100 g BW) | | | | | | | | |
| | **Male** (n = 9–10) | | | | | | | | |
| | Mean (SD) | 0.12 (0.02) | | 0.13 (0.01) | | 0.12 (0.02) | | 0.14 (0.01) | |
| | % 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)<br>Rats, Crj:CD(SD)IGS<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11<br><br>**Data quality:**[e]<br>High (1.2) | **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.
[a]F1 and F2 offspring doses presented as mean maternal gestational and lactational F0 and F1 doses, respectively.
[b]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[c]Exact number of animals examined per dose group was unclear in the published paper.

[d]TWAs 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 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.
[e]Based on OPPT data evaluation criteria

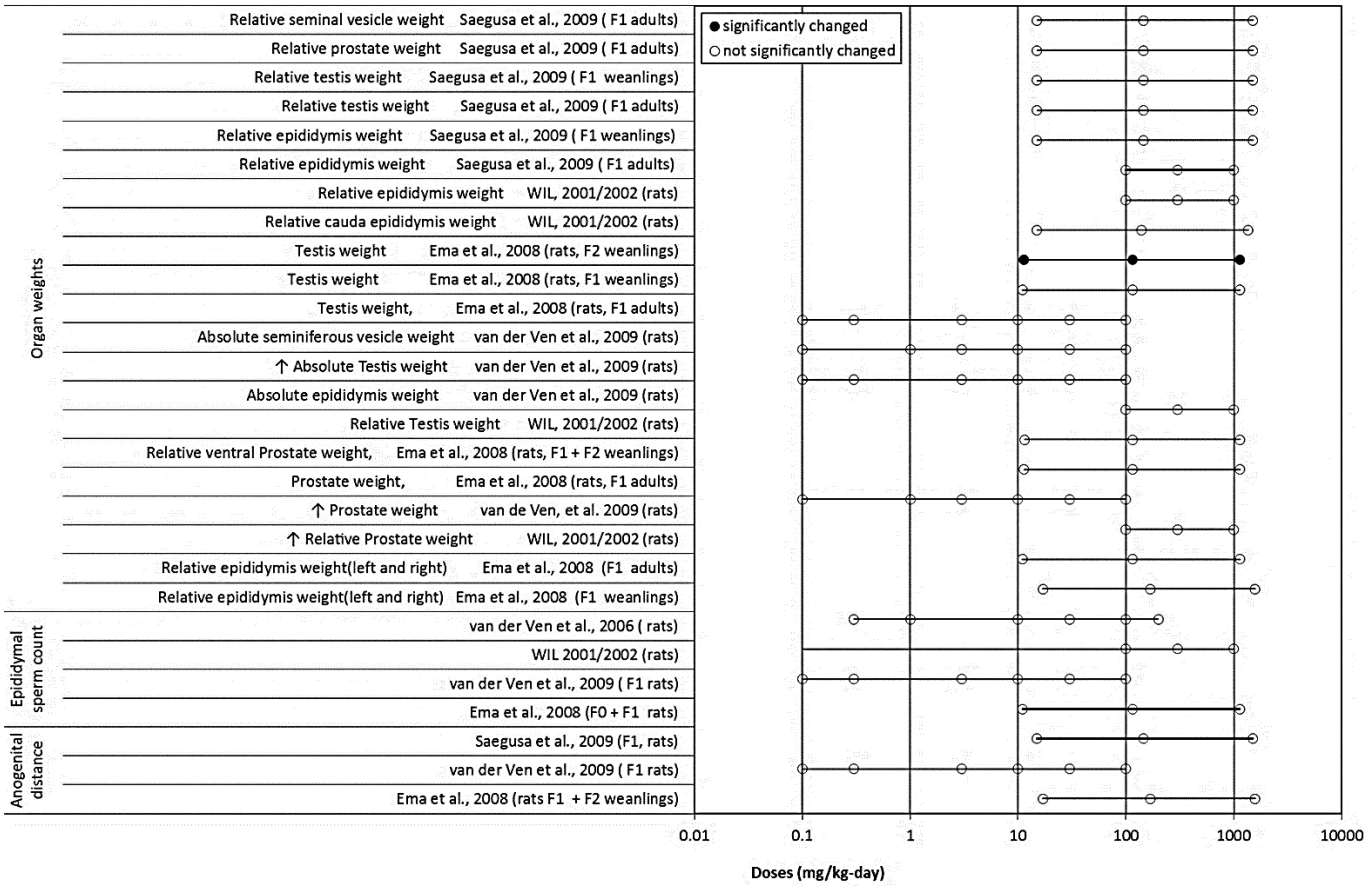



**Figure 1-5. Exposure response array of male reproductive system effects following oral exposure. All studies scored a High in data quality evaluation.**

### 1.3.2.1 Mechanistic Evidence

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

## 1.4 Developmental Effects

### 1.4.1 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).

### 1.4.2 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 | | | | |
|---|---|---|---|---|---|
| *Fetal and early postnatal survival* | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning through necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/lactation<br><br>**Data quality:**[f]<br>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)<br>Rats, Crj:CD(SD)IGS<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non- | **Doses** (mg/kg-d)[c] | | | | |
| | | **0** | **15** | **146** | **1,505** |
| | **Number of live pups** | | | | |
| | **Female, F0** (n = 10 litters) | | | | |
| | Mean (SD) | 13.0 (1.8) | 13.0 (1.6) | 11.6 (1.6) | 12.9 (1.4) |
| | % of control[b] | – | 0% | −11% | −1% |

| Reference and study design | Results | | | | |
|---|---|---|---|---|---|
| exposure period through PNW 11<br><br>**Data quality:**[f]<br>High (1.2) | | | | | |
| *Body weight* | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning through necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/lactation<br><br>**Data quality:**[f]<br>High (1.0) | **Doses** (mg/kg-d) | | | | |
| | **F1 offspring**[a] | **0** | **17** | **168** | **1,570** |
| | **F2 offspring**[a] | **0** | **15** | **139** | **1,360** |
| | **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) | | | | |
| | 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) | | | | |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 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)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating<br>F1: continuous maternal exposure throughout | **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] | | | | | | | | |
| | 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] ** | | | | | | | | |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data quality:**[f]<br>High (1.2) | 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)<br>Rats, Crj:CD(SD)IGS<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11[e]<br><br>**Data quality:**[f]<br>High (1.2) | **Doses** (mg/kg-d)[c] | **0** | **15** | **146** | **1,505** | | | | |
| | **Pup weight** (g) | | | | | | | | |
| | **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% | | | | |
| | **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) | | | | | | | | |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 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)<br>Mice, BALB/c<br>Females only<br>Diet<br>28-d exposure starting on PND 26<br><br>**Data quality:**[f]<br>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% | | | |
| *Developmental markers* | | | | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning through necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/lactation<br><br>**Data quality:**[f]<br>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). | | | | | | | |
| *Bone measures* | | | | | | | | |
| van der Ven et al. (2009)<br>Rats, Wistar | **Doses** (mg/kg-d) | | | | | | | |
| | | **0** | **0.1** | **0.3** | **1** | **3** | **10** | **30** | **100** |
| | **Trabecular bone mineral density, tibia** (mg/cm$^3$) | | | | | | | |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Diet<br>One generation<br><br>F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating<br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data quality:**[f]<br>High (1.2) | **Male, F1, PNW 11** (n = 4−5) | | | | | | | |
| Mean (SD) | 145 (25) | 143 (20) | 154 (23) | 167 (16) | 134 (36) | 146 (25) | 156 (20) | 167 (11) |
| % of control[b] | − | −1% | 6% | 15% | −8% | 1% | 8% | 15% |
| **Female, F1, PNW 11** (n = 5)** | | | | | | | | |
| Mean (SD) | 294 (19) | 268 (27) | 253 (30) | 231 (35) | 245 (31) | 227 (28) | 200 (31) | 234 (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.
[a]F1 and F2 offspring doses presented as mean maternal gestational and lactational F0 and F1 doses, respectively.
[b]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[c]TWA 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 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.
[d]Exact 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).
[f]Based on OPPT data evaluation criteria

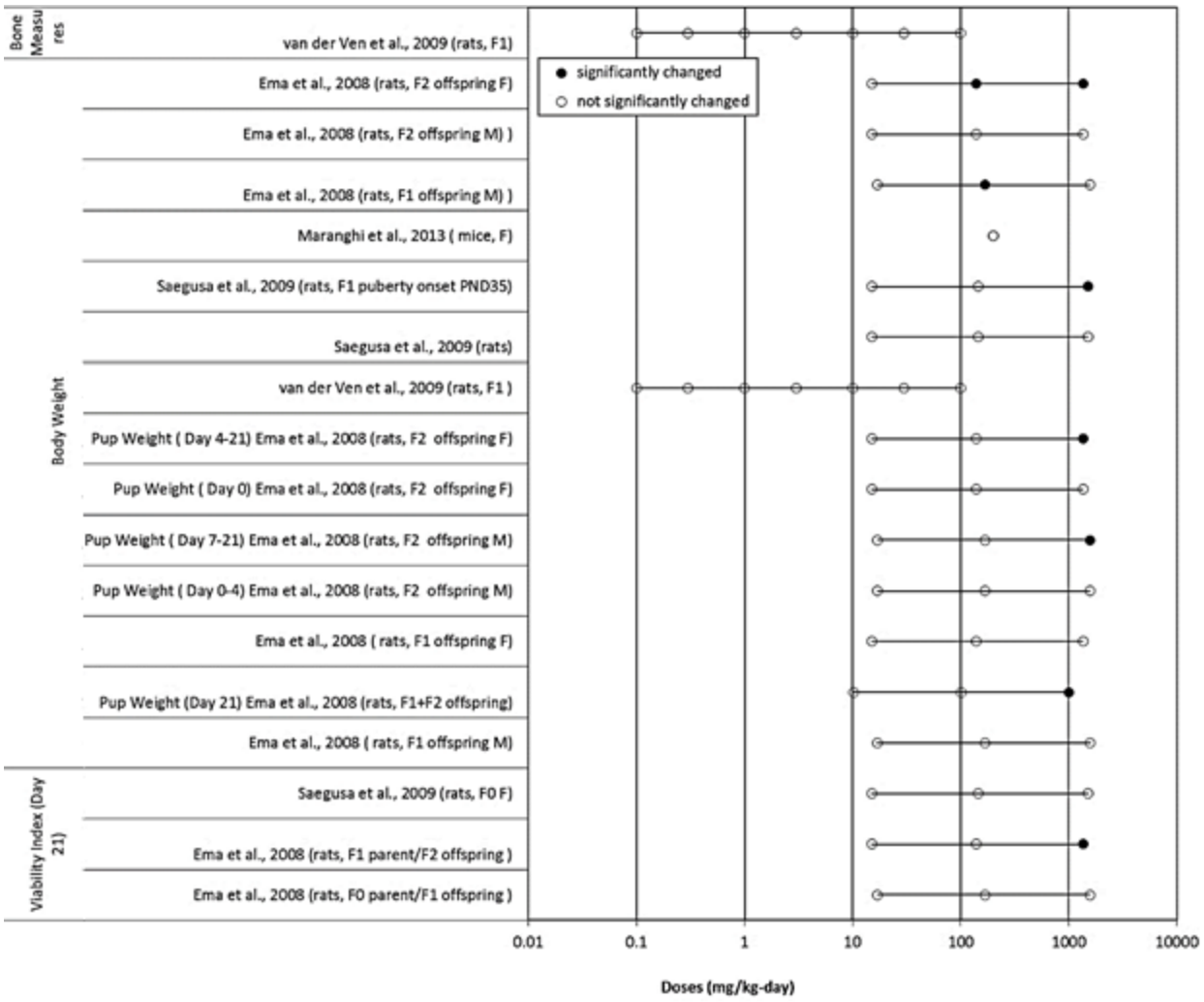



**Figure 1-6. Exposure response array of developmental effects following oral exposure. All studies scored High in data quality evaluation.**

### 1.4.3 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

### 1.5.1 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.

### 1.5.2 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.

#### 1.5.2.1 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 ≥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.

### 1.5.2.2 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 |
|---|---|
| *Studies in infants and children, neurodevelopment* | |
| Roze et al. (2009) (the Netherlands, COMPARE cohort, 2001−2002 at baseline)<br>**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<br>**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<br>Median 0.8 (range: 0.3−7.5) ng/g lipids<br>**Effect measures:**<br>Neuropsychological tests (references for procedure provided)<br>• Movement ABC test battery for motor performance (coordination, fine motor skills)<br>• Developmental Coordination Disorder Questionnaire for behavior<br>• Wechsler Preschool and Primary Scale of Intelligence, Revised for intelligence (total, verbal, performance)<br>• Neuropsychological Assessment (NEPSY-II) for visual perception, visuomotor integration, inhibitory control<br>• Rey's Auditory Verbal Learning test (verbal memory)<br>• Test of Everyday Attention for Children (attention)<br>Behavioral tests (references for procedure provided)<br>• Child Behavior Checklist and Teacher's Report Form<br>• Attention Deficit/Hyperactivity Disorder questionnaire<br>**Analysis:** Pearson correlation (for normally distributed variables) or Spearman's rank correlation (for non-normally distributed variables)<br><br>**Data quality:**<br>Medium (1.8) | Correlations between lipid-adjusted HBCD and outcome measure adjusted for socioeconomic status (maternal education), HOME score, and sex<br><br>*Neuropsychological measure* — *Correlation coefficient*<br>Coordination — 0.290 ($p < 0.05$)<br>Total intelligence — 0.393 ($p < 0.05$)<br>Verbal intelligence — 0.479 ($p < 0.01$)<br><br>(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.)<br><br>Results for correlations between HBCD and other neuropsychological and behavioral outcomes were not shown, but were stated to be not statistically significant ($p > 0.10$). |
| *Studies in adolescents, neurodevelopment* | |
| Kiciński et al. (2012) (Belgium, 2008−2011) | Beta (95% CI)[b] |

| | | |
|---|---|---|
| **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")<br>**Exposure measures:** Serum samples, HBCD >75% were less than the LOQ (LOQ = 30 ng/L); Median <30 ng/L (range: <LOQ−234) ng/L<br>**Effect measures:** Neurobehavior (Neurobehavioral Evaluation System, NES-3), computerized battery (references for procedure provided)<br>Continuous Performance test (attention)<br>Digit-Symbol test (visual scanning and information processing)<br>Digit Span test (working memory)<br>Finger Tapping (motor function)<br>**Analysis:** Regression models (linear or negative binomial depending on outcome)<br><br>**Data quality:**[a]<br>Medium (1.9) | 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) |
| | 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.<br><br>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. | |

[a]Based on OPPT data evaluation criteria
[b]Beta 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 | | | | |
|---|---|---|---|---|---|
| *Neurodevelopmental milestones* | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning through necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/ lactation | **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) |

| Reference and study design | Results | | | | |
|---|---|---|---|---|---|
| **Data quality:**[d] High (0) | % 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 Gavage<br><br>F1: Continuous maternal exposure throughout gestation | **Doses** (mg/kg-d) | **0** | **3** | **10** | **30** |
| | **Age at which 85% of pups could perform righting reflex** | | | | |
| | **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. | | | | |
| **Data quality:**[d] Medium (2)* | **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. | | | | |
| *Executive function and locomotor activity* | | | | | |
| Ema et al. (2008) Rats, CRL:CD(SD) Diet | **Doses** (mg/kg-d) | | | | |
| | **Male, F1** | **0** | **11** | **115** | **1,142** |
| | **Female, F1** | **0** | **14** | **138** | **1,363** |

| Reference and study design | Results | | | | |
|---|---|---|---|---|---|
| Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning until necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/ lactation<br><br>**Data quality:**[d]<br>High (1.0) | **Locomotor activity** | | | | |
| | **Male, F1, PNW 4** (n = 10) | | | | |
| | | Mean (SD)<br>% 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)<br>% of control[b] | | | |
| | 0−10 min | 196.9 (75.8) | 194.1 (112.7) | 176.7 (93.8) | 172.6 (101.9) |
| | | – | −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)<br>% 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) |

| Reference and study design | Results | | | |
|---|---|---|---|---|
| | – | 11% | 2% | −29% |
| | **Female, F1, PNW 6** (n = 10) | | | |
| | | Mean (SD)<br>% 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)<br>Rats, Long-Evans<br>Gavage<br><br>F1: Continuous maternal exposure throughout gestation<br><br>Go/no-go task: animals tested on PNM 14 and 21<br><br>RR task animals tested on PNM 11 and 19<br><br>**Data quality:**[d]<br>Medium (2)* | **Doses** (mg/kg-d) | | | |
| | **0** | **3** | **10** | **30** |
| | **Go/no-go task** (% hits) | | | |
| | **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% |
| | **Random ratio (RR) task** (responses per minute) | | | |
| | **Males and females, F1** (n = 4) | | | |
| | | Mean (SD)<br>% 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.<br>Go/no-go task: hit defined as lever press behavior during a “go” trial.<br>RR task: Different schedules (*e.g.*, RR1, RR2…) correspond to the average number of lever presses between reinforcements. | | | |
| Eriksson et al. (2006)<br>Mice, NMRI<br>Gavage | **Doses** (mg/kg) | | | |
| | **0** | | **0.9** | **13.5** |
| | **Horizontal locomotion** (beam hits) | | | |
| | **Male, F1, PNM 3** (n = 10) | | | |

| Reference and study design | Results | | | |
|---|---|---|---|---|
| F1: single dose on PND 10<br><br>Males only<br><br>**Data quality:**[d]<br>Medium (2)* | | | Mean (SD)<br>% 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)<br>% 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)<br>% 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<br>% 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.<br>Morris water maze: error data not shown. Day 5, platform relocated. | | | |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Other neurological effects* | | | | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning until necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/ lactation<br><br>**Data quality:**[d]<br>High (1.0) | **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 brain weight** (mg) | | | | | | | |
| | **Male, F1 PND 26** (n = 17–23) | | | | | | | |
| | Mean (SD) | 1.64 (0.09) | 1.66 (0.05) | 1.62 (0.07) | 1.55* (0.06) | | | |
| | % 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) | 1.51* (0.06) | | | |
| | % 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) | 2.11* (0.07) | | | |
| | % of control[b] | – | 2% | 0% | −3% | | | |
| | **Female, F1 adult** (n = 13–22) | | | | | | | |
| | 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)<br>Rats, Wistar<br>Diet<br><br>F0: exposure started 10 wks (male) or 2 wks (female) prior to mating<br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning until sacrifice (~PNW 20)<br><br>**Data quality:**[d]<br>High (1.3) | **Doses** (mg/kg-d) | | | | | | | |
| | | **0** | **0.1** | **0.3** | **1** | **3** | **10** | **30** | **100** |
| | **BAEPs, click threshold** (dB) | | | | | | | | |
| | **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 for males were digitized from figure. | | | | | | | | |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **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)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating<br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data quality:**[d]<br>High (1.2) | **Doses** (mg/kg-d) | **0** | **0.1** | **0.3** | **1** | **3** | **10** | **30** | **100** |
| | **Absolute brain weight** (g) | | | | | | | | |
| | **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% |
| | **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.
[a]F1 and F2 offspring doses presented as mean maternal gestational F0 and F1 doses, respectively.
[b]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[c]Exact number of animals examined per dose group was unclear based on the published paper.
[d]Based 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

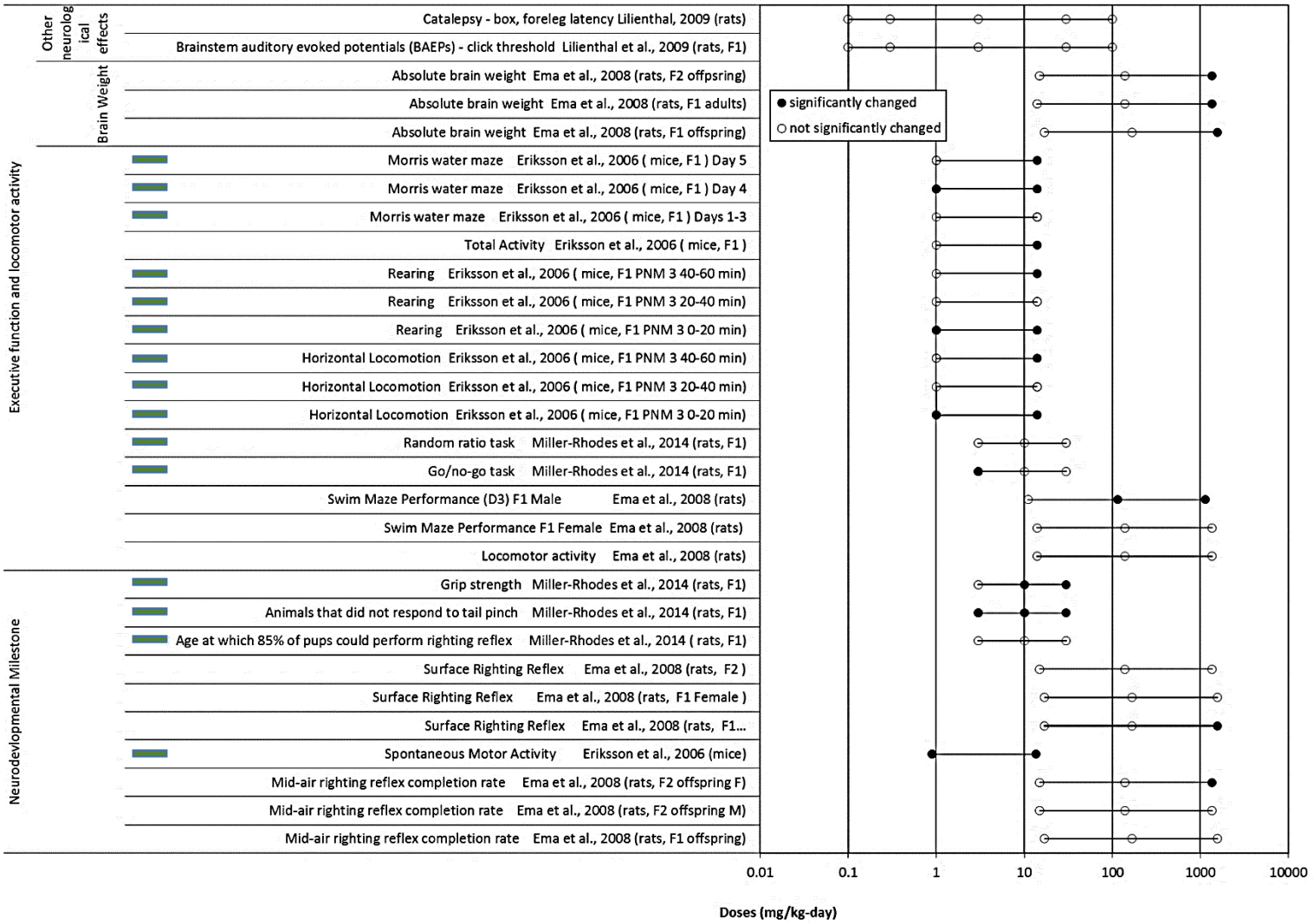



**Figure 1-7. Exposure response array of nervous system effects following oral exposure. Lilienthal et al. (2009) and Ema et al. (2008) scored a High in data quality evaluation. Miller-Rhodes et al. (2014) and Eriksson et al. (2006) scored a Medium (indicated with ▬).**

### 1.5.3 Mechanistic Evidence

#### 1.5.3.1 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).

#### 1.5.3.2 Calcium Homeostasis

Several studies suggest that HBCD may alter calcium ($Ca^{2+}$) homeostasis in the brain by affecting three types of calcium transporters: sarco-endoplasmic reticulum $Ca^{2+}$-dependent ATPase (SERCA) pumps (Al-Mousa and Michelangeli, 2014, 2012), ligand-gated $Ca^{2+}$ channels (LGCC) (Reistad et al., 2006), and voltage-gated $Ca^{2+}$ channels (VGCC) (Dingemans et al., 2009). Within neurons, $Ca^{2+}$ 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 $Ca^{2+}$ levels are restored via active transport across the cell membrane or sequestration into subcellular compartments. Tight regulation of $Ca^{2+}$ is critical as both excess and insufficient levels can adversely affect numerous cellular processes.

SERCA uses ATP to actively transport excess Ca2+ from the cytosol into intracellular compartments to regulate protein synthesis and neurotransmitter release (Neher and Sakaba, 2008; Rodriguez et al., 2001). HBCD increased intracellular Ca2+ 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 Ca2+ affinity conformation (Al-Mousa and Michelangeli, 2014). Exposure of PC12 cells to either the technical mixture or individual HBCD isomers reduced Ca2+ influx through VGCCs, but did not affect resting intracellular Ca2+ 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 Ca2+ 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 Ca2+ channel in neurotoxicity. Although no significant changes in intracellular Ca2+ calcium were reported, this was the only study that measured Ca2+ 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).

#### 1.5.3.3 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 Fonnum, 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.

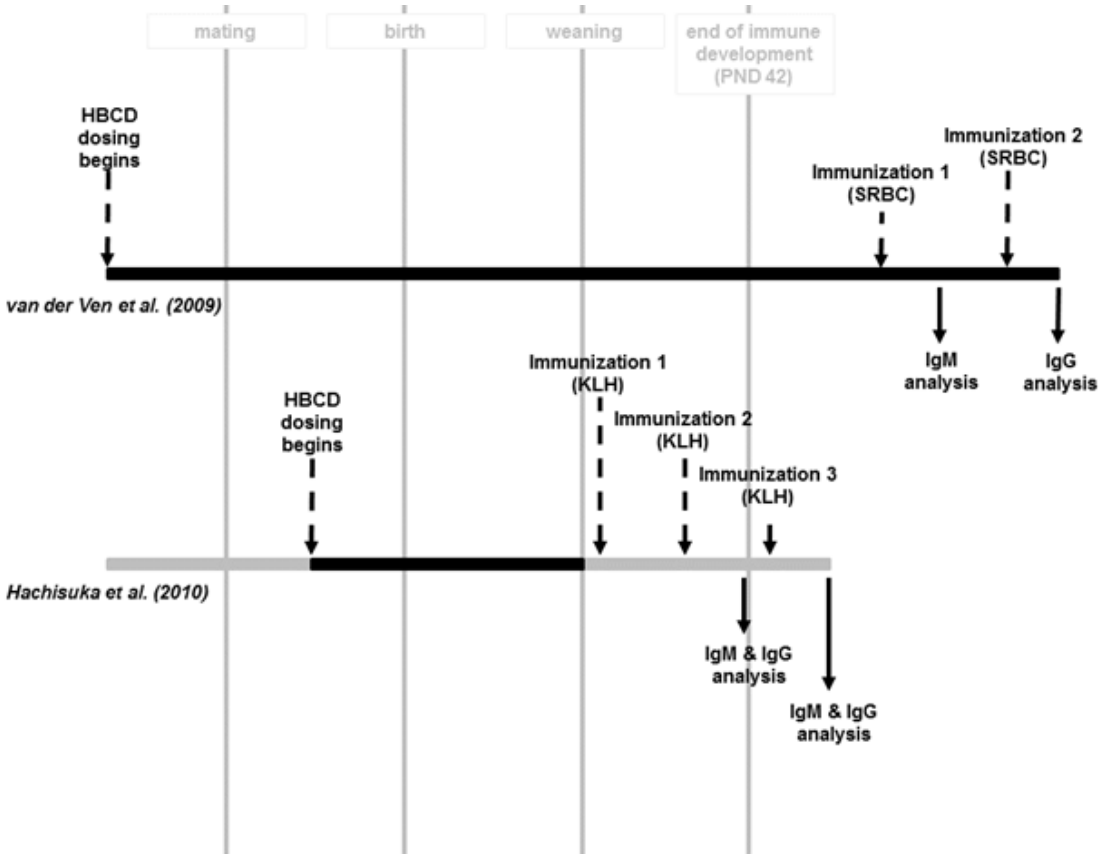
Discussion of immune-related effects of HBCD is organized first by age of exposure (*i.e.*, developmental or adult) and second by the type of endpoint evaluated (*i.e.*, functional or observational). Exposure timing is an important factor that may influence the effect of chemical exposure on immune function, 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 1-8), 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 (≤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)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data quality:**[c]<br>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)<br>Rats, SD:IGS<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11<br><br>**Data quality:**[c]<br>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.
[a]Percent change compared to control calculated as: (treated value − control value)/control value × 100.

[b]TWAs 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.
[c]Based 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 | | | | |
|---|---|---|---|---|---|
| *Organ weight* | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning until necropsy<br>F1/F2 offspring: continuous maternal exposure throughout gestation/lactation<br><br>**Data quality:**[e]<br>High (1.0) | **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) |
| | % change[b] | – | −1% | 8% | −7% |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **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)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>**Data quality:**[e]<br>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)<br>Rats, SD:IGS<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 | **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% | | | | |
| | **Male, F1, PNW 11** | | | | | | | | |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| followed by an 8-wk recovery period through PNW 11<br><br>Only males evaluated<br><br>**Data quality:**[e] Medium (1.9) | 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% | | | |
| *Hematology* | | | | | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: maternal exposure throughout gestation/lactation; dietary exposure post weaning until necropsy<br><br>**Data quality:**[e] High (1.0) | **Doses** (mg/kg-d) | | | | | | | |
| | **Male, F1** | **0** | **11** | **115** | **1,142** | | | |
| | **Female, F1** | **0** | **14** | **138** | **1,363** | | | |
| | **Lymphocyte fraction** (%) | | | | | | | |
| | **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)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11<br><br>Only males evaluated<br><br>**Data quality:**[e] High (1.2) | **Doses** (mg/kg-d) | **0** | **0.1** | **0.3** | **1** | **3** | **10** | **30** | **100** |
| | **Basophil cell count in blood** ($\times 10^9$/L) | | | | | | | | |
| | **Male, F1** (n = 3−4)** | | | | | | | | |
| | 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** ($\times 10^9$/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% |
| Hachisuka et al. | **Doses** (mg/kg-d)[c] | | | | | | | | |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| (2010)<br>Rats, SD:IGS<br>Diet<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11<br><br>Only males evaluated<br><br>**Data quality:**[e]<br>Medium (1.9) | | **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** ($\times 10^2/\mu L$) | | | | | | | |
| | **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)<br>Rats, Wistar<br>Diet<br>One generation<br><br>F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 | **Male, F1**<br>**Female, F1** | **0** | **0.1** | **0.3** | **1** | **3** | **10** | **30** | **100** |
| | **WBC count in bone marrow** ($\times 10^9$/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) |
| | % change[a] | – | 11% | 9% | 13% | 22% | 13% | 14% | 43% |

| Reference and study design | Results | | | |
|---|---|---|---|---|
| **Data quality:**[e] High (1.2) | **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<br><br>F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11<br><br>**Data quality:**[e] Medium (1.9) | **Doses** (mg/kg-d)[c] | | | |
| | **Male, F1**<br>**Female, F1** | **0** | **15** | **146** | **1,505** |
| | **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 NKRP1A+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 |
| | **Female, F1, PNW 11** (n = 10) | | | |

| Reference and study design | Results | | | |
|---|---|---|---|---|
| | 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.
[a]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[b]F1 and F2 offspring doses presented as mean maternal gestational F0 and F1 doses, respectively.
[c]TWAs 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.
[d]Not measured; only control and high-dose values reported.
[e]Based 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 | | | |
|---|---|---|---|---|
| *Organ weight* | | | | |
| Ema et al. (2008)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: dietary exposure post weaning until necropsy<br>F1/F2 offspring: continuous maternal | **Doses** (mg/kg-d) | | | |
| | **Male, F0** | **0** | **10** | **101** | **1,008** |
| | **Female, F0** | **0** | **14** | **141** | **1,363** |
| | **Absolute spleen weight** (mg) | | | | |
| | **Male, F0** (n = 22−24) | | | | |
| | 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% |
| | **Absolute thymus weight** (mg) | | | | |
| | **Male, F0** (n = 22−24) | | | | |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| exposure throughout gestation/lactation<br><br>**Data quality:**[b]<br>High (1.0) | 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)<br>Rats, Wistar<br>Gavage<br>28-d exposure starting on PNW 11<br><br>**Data quality:**[b]<br>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)<br>Rats, CRL:CD(SD)<br>Diet<br>Two generation<br><br>F0: exposure started 10 wks prior to mating<br>F1: maternal exposure throughout gestation/lactation; dietary exposure post weaning until necropsy<br><br>**Data quality:**[b]<br>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) | | | | | | | | |
| | Mean (SD) | 21.68 (8.08) | | 10.56* (4.19) | | 16.84 (9.19) | | 23.28 (8.13) | |
| | % change[a] | – | | −51% | | −22% | | 7% | |

| Reference and study design | Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **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)<br>Rats, Wistar<br>Gavage<br>28-d exposure starting on PNW 11<br><br>**Data quality:**[b]<br>High (1.3) | **Doses** (mg/kg-d) | | | | | | | |
| | **Male** | **0** | **0.3** | **1** | **3** | **10** | **30** | **100** | **200** |
| | **Lymphocyte cell fraction in blood** (%) | | | | | | | |
| | **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) |
| | % change[a] | – | 0% | −4% | −4% | −3% | 0% | −5% | −1% |
| *Histopathology* | | | | | | | | |
| van der Ven et al. (2006)<br>Rats, Wistar<br>Gavage<br>28-d exposure starting on PNW 11<br><br>**Data quality:**[b]<br>High (1.3) | **Doses** (mg/kg-d) | | | | | | | |
| | | **0** | **0.3** | **1** | **3** | **10** | **30** | **100** | **200** |
| | **CD4 (Th) cells per spleen** (cells ×$10^7$) | | | | | | | |
| | **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) |
| | % change[a] | – | 9% | −5% | −19% | −25% | −36% | −20% | −29% |
| | **Total immune cells per spleen** (cells ×$10^7$) | | | | | | | |
| | **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.
[a]Percent change compared to control calculated as: (treated value − control value)/control value × 100
[b]Based on OPPT data evaluation criteria.

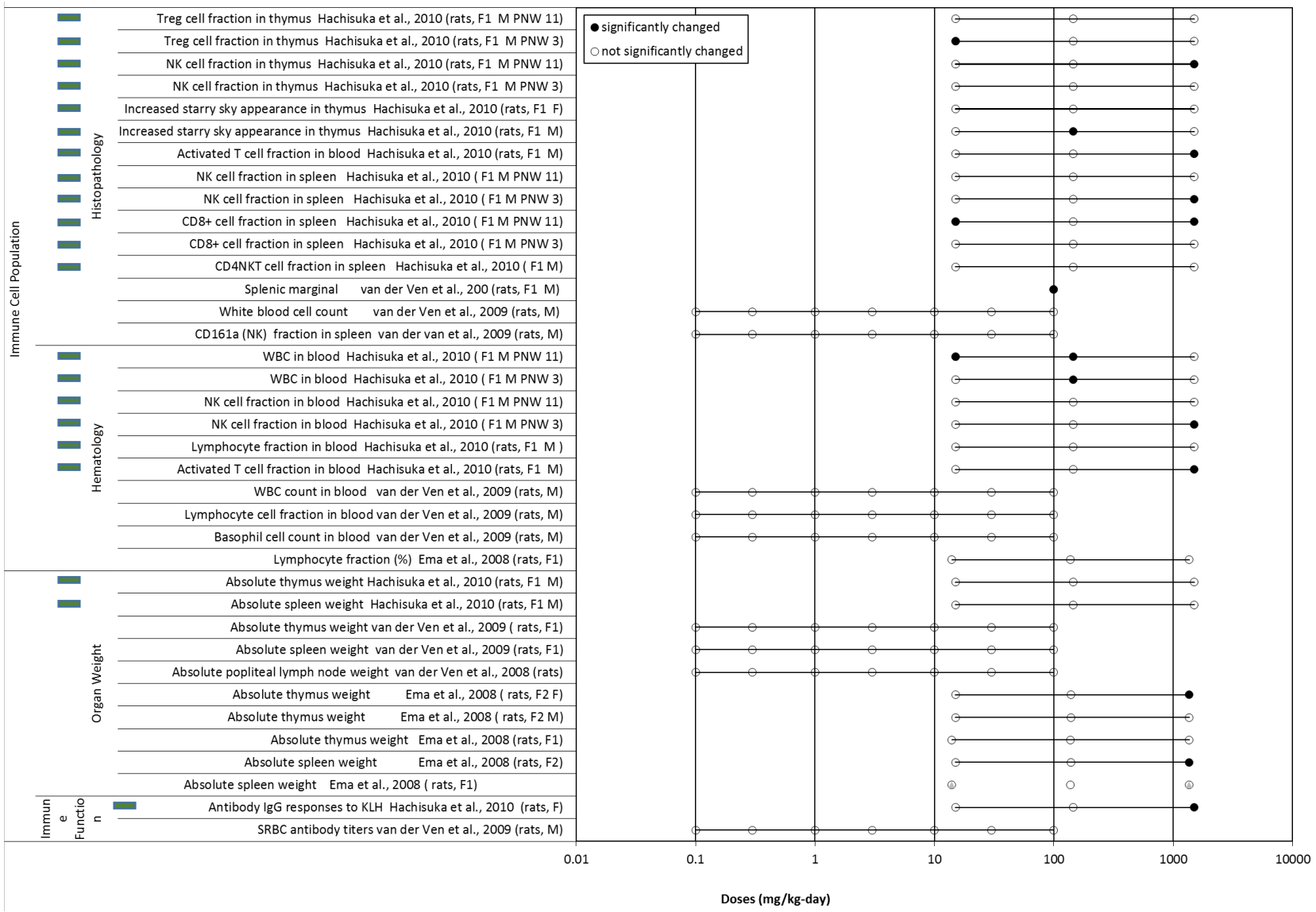



**Figure 1-9. Exposure response array of immune system following oral exposure. Most data was from Hachisuka et al. (2010), which scored a Medium in data quality evaluation (indicated with ▬). All other studies scored a High.**

### 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-1β, 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 brochial 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 μg/mL) increased cell proliferation and expression of a dendritic activation marker, DEC205. Bone marrow-derived dentritic 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 (International, 1990; Litton, 1990; Pharmakologisches, 1990; Zeiger et al., 1987; Ameribrom 1990). Negative results were also obtained in (Gsri, 1978), (IBT Labs, 1990)and (Huntingdon Research, 1990), however these studies scored Unacceptable. Among the few assays performed to determine the genotoxicity of HBCD in eukaryotic systems, one in yeast (Litton, 1990) and one detecting chromosomal aberrations in human peripheral lymphocytes in vitro (Microbiological, 1996) were negative, even when tested at cytotoxic concentrations. A single in vivo mouse micronucleus test following intraperitoneal (i.p.) injections of HBCD (Basf, 2000) was also negative, however the full study was unavailable for data quality review.

**Table 1-13. Summary of genotoxicity studies of HBCD**

| Test/species/strain/ route | Test doses (per plate)[a] | Results[b] | | Notes | Reference | Data Quality |
|---|---|---|---|---|---|---|
| | | −S9 | +S9 | | | |
| **Prokaryotic systems, in vitro** | | | | | | |
| *S. typhimurium* TA98, TA100, TA1535, TA1537 | 50−5,000 µg (HBCD bottoms) in acetone | + (TA1535 and 100 only) | + (TA100 only) | 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) | Medium |
| *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | 50 µg (421−32B) (solvent not reported) | − | − | | Litton (1990) | Medium |
| *S. typhimurium* TA98, TA100, TA1535, TA1537 | 2−1,000 µg (GLS-S6-41A) in DMSO | − | − | | Gsri (1978) | Un-acceptable |
| *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | 100−10,000 µg in DMSO | − | − | Doses ≥1,000 µg were insoluble. | Zeiger et al. (1987) | High |
| *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | 250 µg (Firemaster, FM-100, Lot 53, white powder) in DMSO | − | − | Doses ≥250 µg were insoluble. | IBT Labs (1990) | Un-acceptable |
| | 1,000 µg (FM-100, Lot 3322, liquid residue) in DMSO | − | + (TA1535 only) | Significant in TA1535 at highest dose only. | | |
| *S. typhimurium* TA98, TA100, TA1537 | 3,000 µg in DMSO | − | − | Doses ≥1,000 µg were partially insoluble. | Pharmakologisches (1990) | High |
| *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | 5,000 µg in DMSO | − | − | No cytotoxicity observed. | SRI International (1990) | High |
| *S. typhimurium* TA92, TA94, | 10,000 µg (Pyroguard SR-103) | − | − | | Ogaswara and Hanafusa (1993) | Not reviewed-full study |

| Test/species/strain/ route | Test doses (per plate)[a] | Results[b] | | Notes | Reference | Data Quality |
|---|---|---|---|---|---|---|
| | | −S9 | +S9 | | | |
| TA98, TA100, TA1535, TA1537 | in DMSO | | | | | not available |
| *S. typhimurium* TA98, TA100, TA1535 | 10,000 µg in DMSO | − | − | Insoluble at 10,000 µg. | Huntingdon Research (1990) | Un-acceptable |
| *S. typhimurium* TA98, TA100, TA1535, TA1537, TA-1538 | 0.25−260 µg in DMSO | − | − | | (Ameribrom 1990) | High |
| **Eukaryotic non-mammalian systems, in vitro** | | | | | | |
| *Saccharomyces cerevisiae* D4 | 50 µg (solvent not reported) | − | − | | Litton (1990) | Medium |
| **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 2nd injection. | Basf (2000) | Not reviewed-full study not available |
| **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) | High |
| Reversion assay CHO/V79/Sp5 and SPD8 Intragenic recombination at *hprt* 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 occurred at doses ≥15 µg/mL in the SPD8 assay and ≥20 µg/mL in the Sp5 assay. Cytotoxicity ($IC_{50}$) | Helleday et al. (1999) | High |

| Test/species/strain/ route | Test doses (per plate)[a] | Results[b] | | Notes | Reference | Data Quality |
|---|---|---|---|---|---|---|
| | | −S9 | +S9 | | | |
| | | | | measured at 0.02−0.03 mM. | | |
| Unscheduled DNA synthesis rat/F344 male/primary hepatocytes | 10 µg/well in acetone (HBCD bottoms) | + | NA | Five highest doses (from 5 µg/well) showed an increased response with dose over solvent control, but only four highest were statistically significant ($\chi^2$). Highest dose (1,000 µg/well) was cytotoxic. | Ethyl (1990a) | Medium |
| Comet assay in L02 human hepatocyte cells | $10^{-13}$ M to 60 µM in DMSO | + | NA | Statistically significant dose-responsive increases in DNA damage observed ≥ 20 µM | An et al. (2013) | High |
| Comet assay in L02 human hepatocyte cells and HepG2 human hepatoma cells | $10^{-7}$ to $10^{-5}$ M in DMSO | + | NA | Statistically significant dose-responsive increases in DNA damage observed in both cells; L02 cells showed significance at lower dose | (Huang et al. 2016) | High |

[a]Lowest effective dose for positive results; highest dose tested for negative results.
[b]+ = positive; ± = 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 (IBT 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 µg/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) as well as comet assays in human hepatocyte L02 and hepatoma HepG2 cells (An et al., 2013; Huang et al. 2016) and each study showed a dose-responsive increase in response. Interestingly a followup study by An et al. (2016) found that pre-incubation of L02 cells with sub-mutagenic doses of HBCD promoted adaptive responses that protect against genotoxic effects of subsequent high doses.

It is noteworthy that in these three studies, the positive results were dose-dependent, observed at nontoxic doses, and in two assays, specific for detecting mutations. However, the tests in bacteria and yeast were predominantly negative along with the single mammalian *in vivo* study (Basf, 2000) were predominantly negative.

# 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, 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.

The effects determined to best represent each of the hazards were identified in Section 1, 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 1-2 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 primary Risk Evaluation document 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 used instead.

#### 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 1-2). 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 primary Risk Evaluation document 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 ≥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).

Despite statistical tests indicating that the datasets were compatible for combining, EPA determined that the F0 and F1 data were not truly independent related datasets. Due to HBCD's bioaccumulation over time, the F1 generation experiences additional continuous exposure compared to F0 animals, and the statistical tests may not account for this confounder. Therefore, the data for increased incidence of non-pregnancy was not considered appropriate for combining, and without statistical significance on either data set alone, the endpoint does not represent a confirmed adverse effect. Nonetheless, the analysis is presented in Section 3.2.3.3 for reference.

### 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).

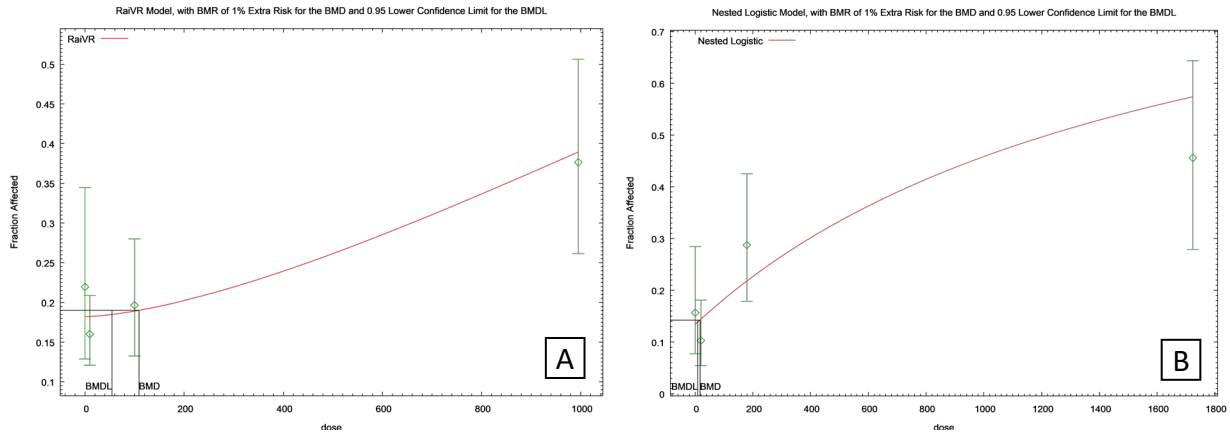



**Figure 2-1. BMD modeling plots of incidence of offspring loss from implantation through PND 4 in F2 offspring rats (A) and incidence of offspring loss from PND 4 post-culling through PND 21 in F2 offspring rats (B) from Ema et al. (2008); BMR = 1% ER (see Appendix D, Figures D-31 and D-33).**

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 primary Risk Evaluation document 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. 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 ± SD (number of animals or litters) | BMR(s) |
|---|---|---|---|---|
| Thyroid | | | | |
| ↓T4<br>Ema et al. (2008) | F0 rats (CRL Sprague-Dawley)/male | 0<br>10<br>101<br>1,008<br><br>TWA of lifetime exposure, F0 | 4.04 ± 1.42 (8)<br>3.98 ± 0.89 (8)<br>2.97 ± 0.76 (8)<br>2.49 ± 0.55 (8) | 10% RD, 15% RD, 20% RD, 1 SD |
| ↓T4<br>Ema et al. (2008) | F0 rats (CRL Sprague-Dawley)/female | 0<br>14<br>141<br>1,363<br><br>TWA of lifetime exposure, F0 | 2.84 ± 0.61 (8)<br>3.14 ± 0.48 (8)<br>3.00 ± 0.77 (8)<br>1.96 ± 0.55 (8) | 10% RD, 15% RD,<br>20% RD, 1 SD |
| ↓T4<br>Ema et al. (2008) | F1 rats (CRL Sprague-Dawley)/female | 0<br>14.3<br>138<br>1,363<br><br>TWA of lifetime exposure, F1 | 3.59 ± 1.08 (8)<br>3.56 ± 0.53 (8)<br>3.39 ± 1.21 (8)<br>2.58 ± 0.37 (8) | 10% RD, 15% RD,<br>20% RD, 1 SD |
| Liver | | | | |
| Relative liver weight<br>Ema et al. (2008) | F1 rats (CRL Sprague-Dawley)/male weanlings, PND 26 | 0<br>16.5<br>168<br>1,570<br><br>TWA of F0 gestational and lactational doses | 4.6 ± 0.37 (23)<br>4.6 ± 0.32 (21)<br>5.05 ± 0.32 (20)<br>6 ± 0.44 (17) | 10% RD, 1 SD |

| Endpoint | Species (strain)/sex | Dose (mg/kg-d)[a] | Incidence [%] or mean ± SD (number of animals or litters) | BMR(s) |
|---|---|---|---|---|
| Relative liver weight<br>Ema et al. (2008) | F1 rats (CRL Sprague-Dawley)/female weanlings, PND 26 | 0<br>16.5<br>168<br>1,570<br><br>TWA of F0 gestational and lactational doses | 4.57 ± 0.35 (23)<br>4.59 ± 0.28 (21)<br>5.02 ± 0.32 (20)<br>6.07 ± 0.36 (14) | 10% RD, 1 SD |
| Relative liver weight<br>Ema et al. (2008) | F1 rats (CRL Sprague-Dawley)/male adults | 0<br>11.4<br>115<br>1,142<br><br>TWA of lifetime exposure, F1 | 3.27 ± 0.18 (24)<br>3.34 ± 0.26 (24)<br>3.37 ± 0.25 (22)<br>3.86 ± 0.28 (24) | 10% RD, 1 SD |
| Relative liver weight<br>Ema et al. (2008) | F1 rats (CRL Sprague-Dawley)/female adults | 0<br>14.3<br>138<br>1,363<br><br>TWA of lifetime exposure, F1 | 4.18 ± 0.42 (22)<br>4.39 ± 0.44 (22)<br>4.38 ± 0.47 (20)<br>5.05 ± 0.50 (13) | 10% RD, 1 SD |
| Relative liver weight<br>Ema et al. (2008) | F2 rats (CRL Sprague-Dawley)/male weanlings, PND 26 | 0<br>14.7<br>139<br>1,360<br><br>TWA of F1 gestational and lactational doses | 4.72 ± 0.59 (22)<br>4.74 ± 0.35 (22)<br>5.04 ± 0.4 (18)<br>6.0 ± 0.25 (13) | 10% RD, 1 SD |
| Relative liver weight<br>Ema et al. (2008) | F2 rats (CRL Sprague-Dawley)/female weanlings, PND 26 | 0<br>14.7<br>139<br>1,360<br><br>TWA of F1 gestational and lactational doses | 4.70 ± 0.27 (21)<br>4.70 ± 0.28 (22)<br>4.94 ± 0.32 (20)<br>5.89 ± 0.44 (13) | 10% RD, 1 SD |
| Relative liver weight and hepatocellular vacuolization<br>WIL Research (2001) | Rats (Sprague-Dawley)/male | 0<br>100<br>300<br>1,000 | 2.709 ± 0.1193 (10)<br>3.175 ± 0.2293 (10)<br>3.183 ± 0.2653 (10)<br>3.855 ± 0.1557 (9) | 10% RD, 1 SD |
| Relative liver weight and hepatocellular vacuolization<br>WIL Research (2001) | Rats (Sprague-Dawley)/female | 0<br>100<br>300<br>1,000 | 2.887 ± 0.2062 (10)<br>3.583 ± 0.2734 (10)<br>3.578 ± 0.3454 (10)<br>4.314 ± 0.2869 (10) | 10% RD, 1 SD |
| Reproductive | | | | |

| Endpoint | Species (strain)/sex | Dose (mg/kg-d)[a] | Incidence [%] or mean ± SD (number of animals or litters) | BMR(s) |
|---|---|---|---|---|
| Primordial follicles<br>Ema et al. (2008)<br>(supplemental) | F1 parental rat (CRL Sprague-Dawley)/female | 0<br>9.6<br>96<br>941<br><br>The F0 adult female gestational doses | 316.3 ± 119.5 (10)<br>294.2 ± 66.3 (10)<br>197.9 ± 76.9 (10)<br>203.4 ± 79.5 (10) | 1% ER, 5% ER, 10% ER |
| Incidence of non-pregnancy<br>Ema et al. (2008) | F0 and F1 parental rats combined (CRL Sprague-Dawley)/female | 0<br>13.3<br>132<br>1,302<br><br>TWA F0, F1 female pre-mating doses | 1/48 [2%]<br>3/48 [6.2%]<br>7/48 [14.5%]<br>7/47 [14.9%] | 5% ER, 10% ER |
| Developmental | | | | |
| Offspring loss at PND 4<br>Ema et al. (2008) | F2 offspring rats (CRL Sprague-Dawley) | 0<br>9.7<br>100<br>995<br><br>The F1 adult female gestational doses | 28/132 [21%]<br>26/135 [19.3%]<br>23/118 [19.5%]<br>47/120 [39.2%] | 1% ER, 5% ER |
| Offspring loss at PND 21<br>Ema et al. (2008) | F2 offspring rats (CRL Sprague-Dawley) | 0<br>19.6<br>179<br>1,724<br>The F1 adult female lactational doses | 11/70 [15.7%]<br>7/70 [10.0%]<br>18/64 [28.1%]<br>32/64 [50.0%] | 1% ER, 5% ER |
| Pup weight during lactation at PND 21<br>Ema et al. (2008) | F2 offspring rats (CRL Sprague-Dawley)/male | 0<br>19.6<br>179<br>1,724<br><br>The F1 adult female lactational doses | 53 ± 12.6 (22)<br>56.2 ± 6.7 (22)<br>54.1 ± 10.1 (18)<br>42.6 ± 8.3 (13) | 5% RD, 10% RD,<br>0.5 SD, 1 SD |
| Pup weight during lactation at PND 21<br>Ema et al. (2008) | F2 offspring rats (CRL Sprague-Dawley)/female | 0<br>19.6<br>179<br>1,724<br><br>The F1 adult female lactational doses | 52 ± 10 (21)<br>52.8 ± 6.6 (22)<br>51.2 ± 10.8 (20)<br>41.6 ± 8.4 (13) | 5% RD, 10% RD,<br>0.5 SD, 1 SD |

[a]Doses 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 models[1] 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 ($\chi 2$ 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 models[2] 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 ($\chi 2$ 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 models[3] 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 ($\chi 2$ p-value ≥ 0.10), the model was fitted to the data assuming constant variance. If Test 2 was rejected ($\chi 2$ 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 $\chi 2$ 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

[1]Unless 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 ≥1; for the Gamma and Weibull models, restrict power ≥1.
[2]Unless otherwise specified, all available BMDS nested dichotomous models were fitted. For the nested Logistic, NCTR, and Rai and van Ryzin models, power ≥1 was applied.
[3]Unless 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 ≥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<br>Intralitter correlations estimated | Litter-specific covariates used<br>Intralitter correlations assumed zero |
| Litter-specific covariates not used<br>Intralitter correlations estimated | Litter-specific covariates not used<br>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. EPA, 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 × 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.

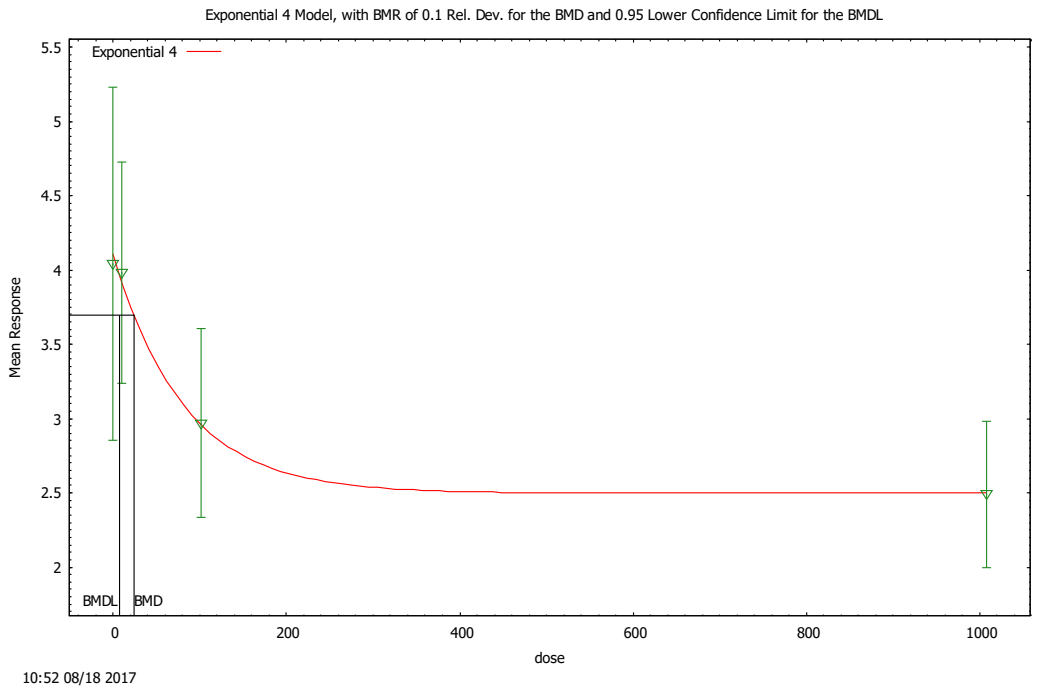
### 3.2.3.1 Thyroid

**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)<br>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)<br>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]<br>Polynomial 3°[e]<br>Polynomial 2°[f]<br>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)<br>Exponential (M3)[b] | 0.0473 | 33.926 | 548 | 376 | 866 | 511 | |
| Exponential (M4)<br>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]<br>Polynomial 3°[e]<br>Polynomial 2°[f]<br>Linear | 0.0418 | 34.174 | 607 | 454 | 906 | 595 | |

[a]Modeled 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.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For 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.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[g]BMD or BMDL computation failed for this model.

Data from Ema et al. (2008)




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:
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * 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 |

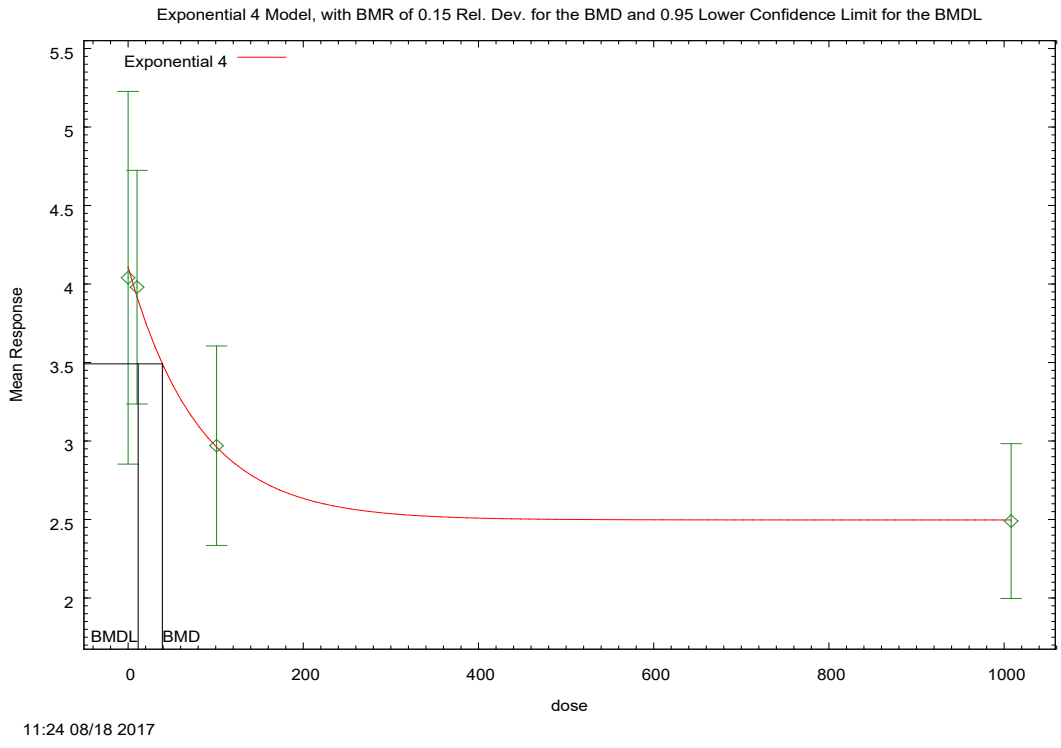
## Likelihoods of Interest

| 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




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:
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * 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) |

## 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 |

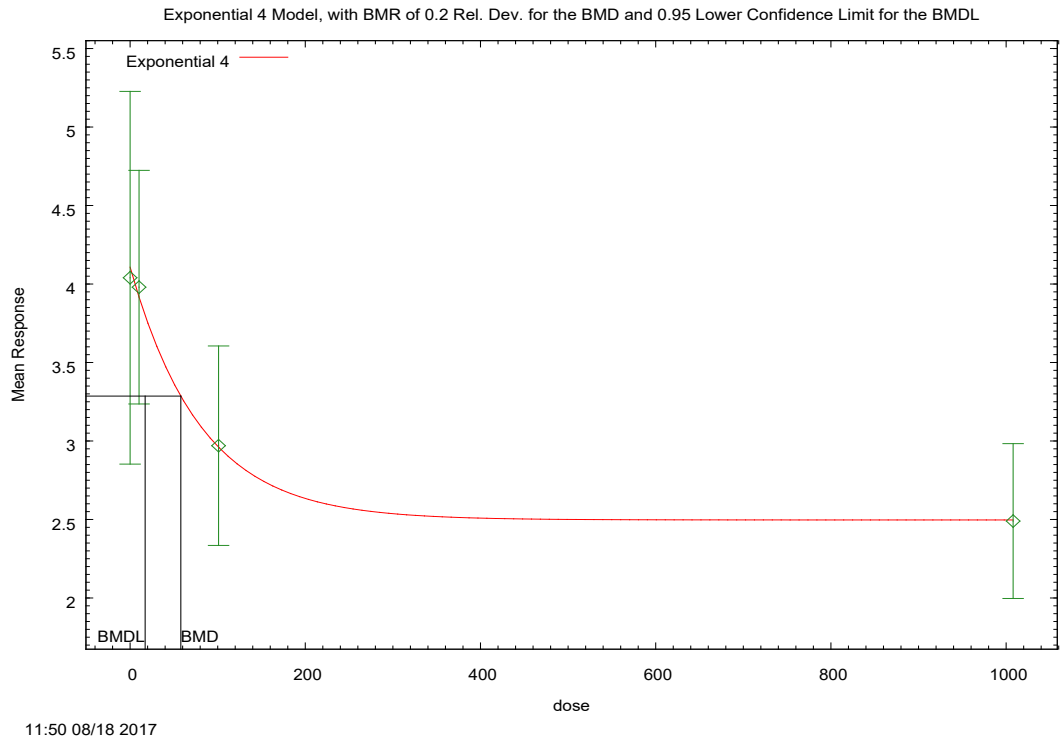
## Likelihoods of Interest

| 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




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:
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * 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 |

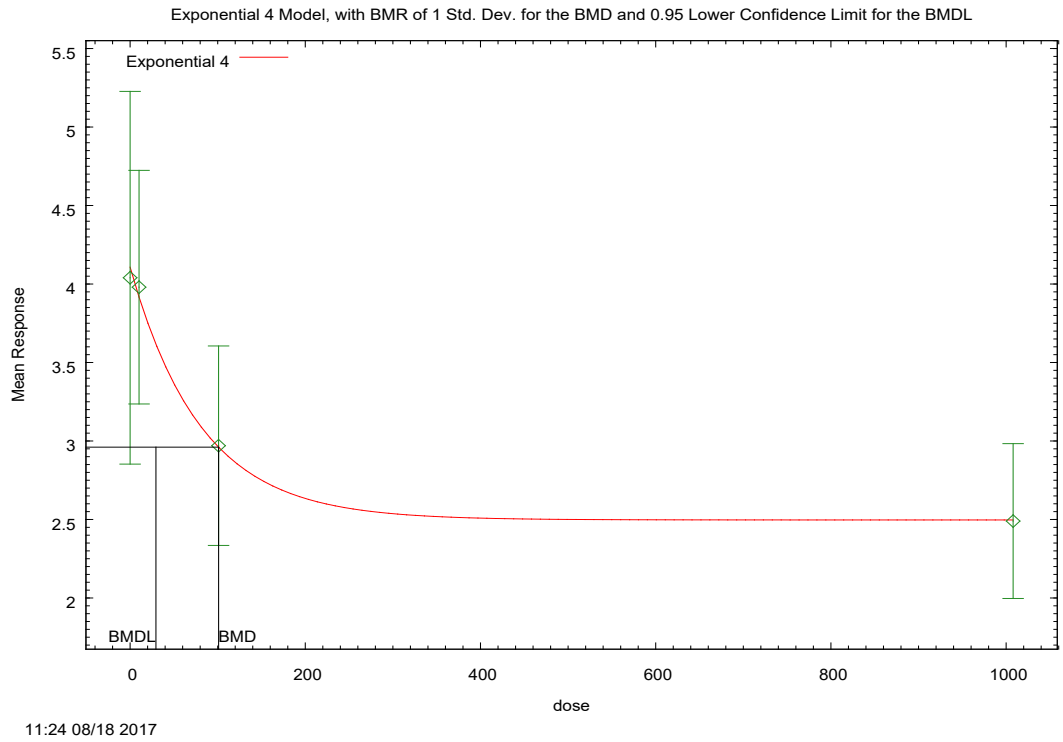
## Likelihoods of Interest

| 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




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

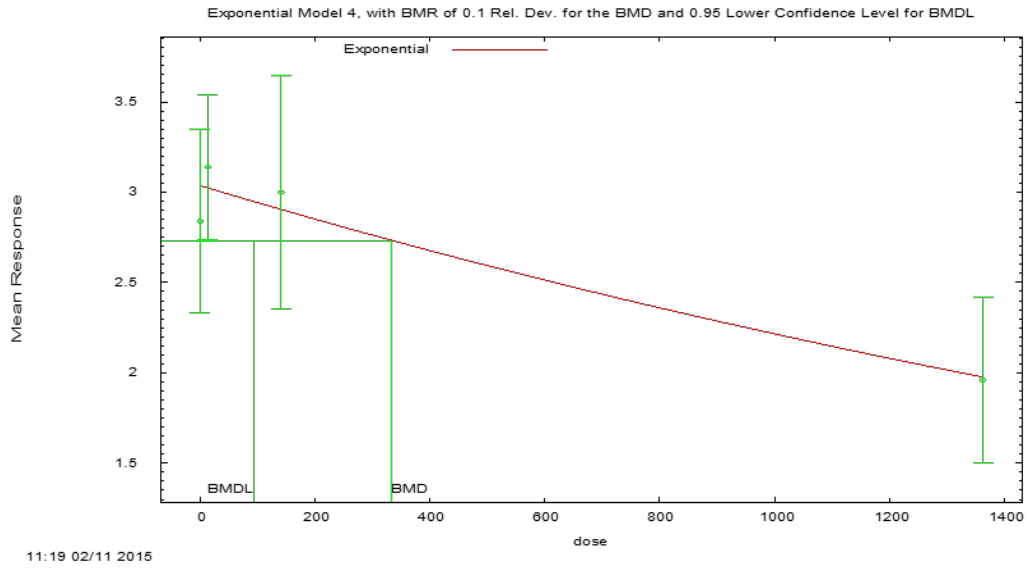
| Test | −2*log (likelihood ratio) | Test df | p-value |
|---|---|---|---|
| Test 1 | 20.65 | 6 | 0.002123 |
| Test 2 | 6.887 | 3 | 0.07559 |
| Test 3 | 1.185 | 2 | 0.553 |
| Test 6a | 0.108 | 1 | 0.7424 |

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 | 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 (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° | 0.582 | 3.3778 | 902 | 816 | 1,032 | 934 | |
| Polynomial 2° | 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° | 0.582 | 3.3778 | 1,136 | 1,028 | 1,126 | 999 | |
| Polynomial 2° | 0.580 | 3.3836 | 1,036 | 586 | 1,021 | 532 | |
| Linear | 0.505 | 3.6625 | 779 | 577 | 751 | 523 | |

[a]Constant 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.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD 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[dose] = a * [c-(c-1) * exp(-b * 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 |

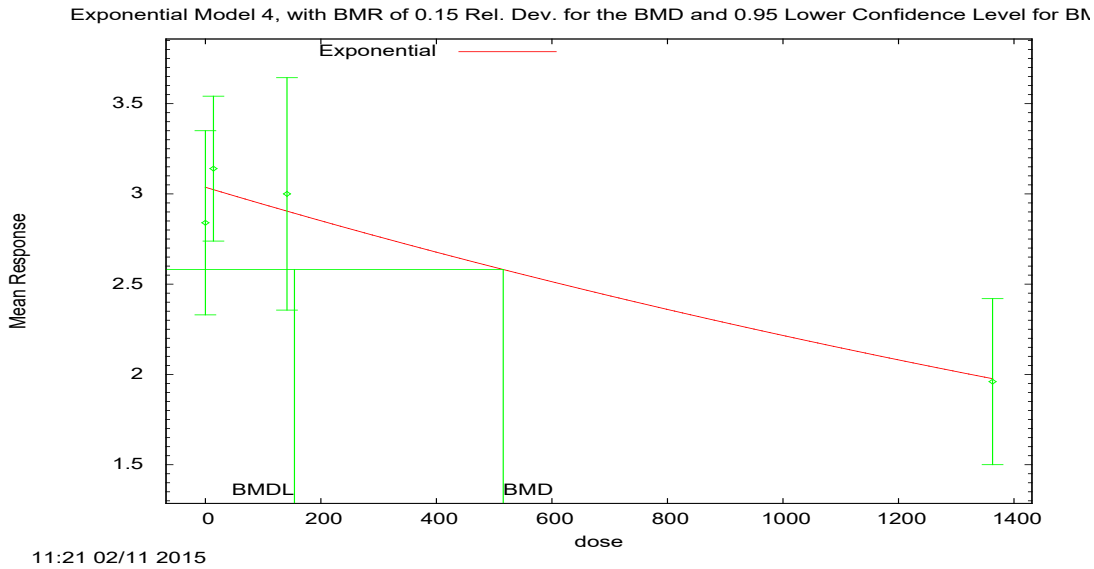
| | | | | | | |
|---|---|---|---|---|---|---|
| 14 | 8 | 3.14 | 3.023 | 0.48 | 0.5857 | 0.5631 |
| 141.3 | 8 | 3 | 2.905 | 0.77 | 0.5857 | 0.4611 |
| 1,363 | 8 | 1.96 | 1.976 | 0.55 | 0.5857 | −0.07911 |

**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 |




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[dose] = a * [c-(c-1) * exp(-b * 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**

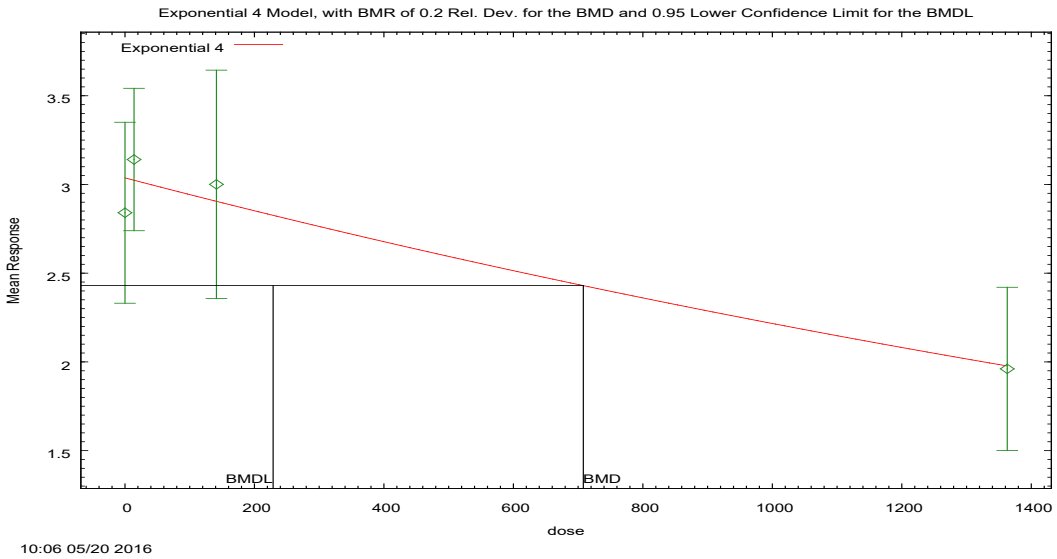
| Dose | N | Observed mean | Estimated mean | Observed SD | Estimated SD | Scaled residuals |
|---|---|---|---|---|---|---|
| 0 | 8 | 2.84 | 3.037 | 0.61 | 0.5857 | −0.9501 |
| 14 | 8 | 3.14 | 3.023 | 0.48 | 0.5857 | 0.5631 |
| 141.3 | 8 | 3 | 2.905 | 0.77 | 0.5857 | 0.4611 |
| 1,363 | 8 | 1.96 | 1.976 | 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[dose] = a * [c-(c-1) * exp(-b * 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 |

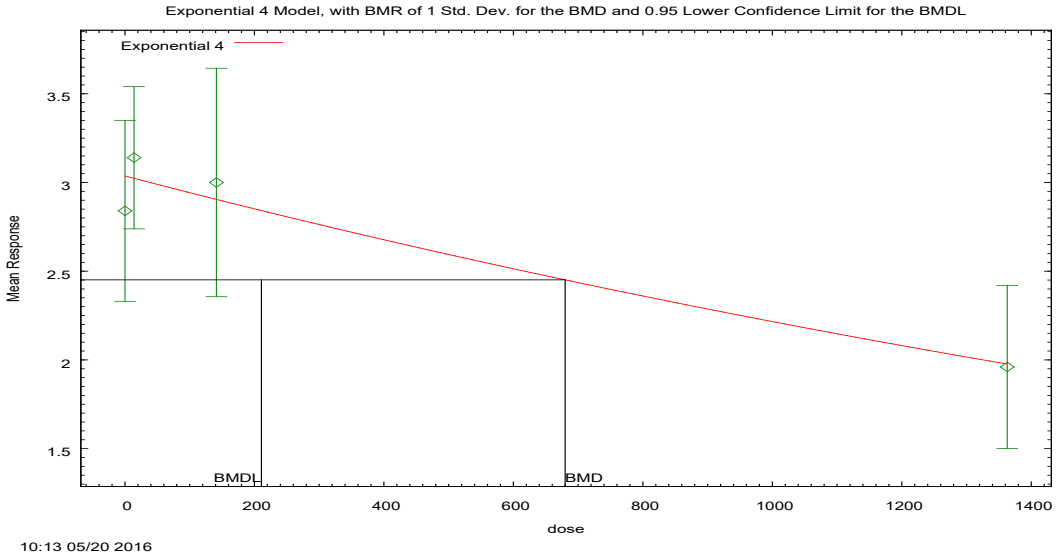
| | | | | | | |
|---|---|---|---|---|---|---|
| 141.3 | 8 | 3 | 2.9 | 0.77 | 0.59 | 0.4611 |
| 1,363 | 8 | 1.96 | 1.98 | 0.55 | 0.59 | −0.07911 |

**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[dose] = a * [c-(c-1) * exp(-b * 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 |
|---|---|---|
| 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 |

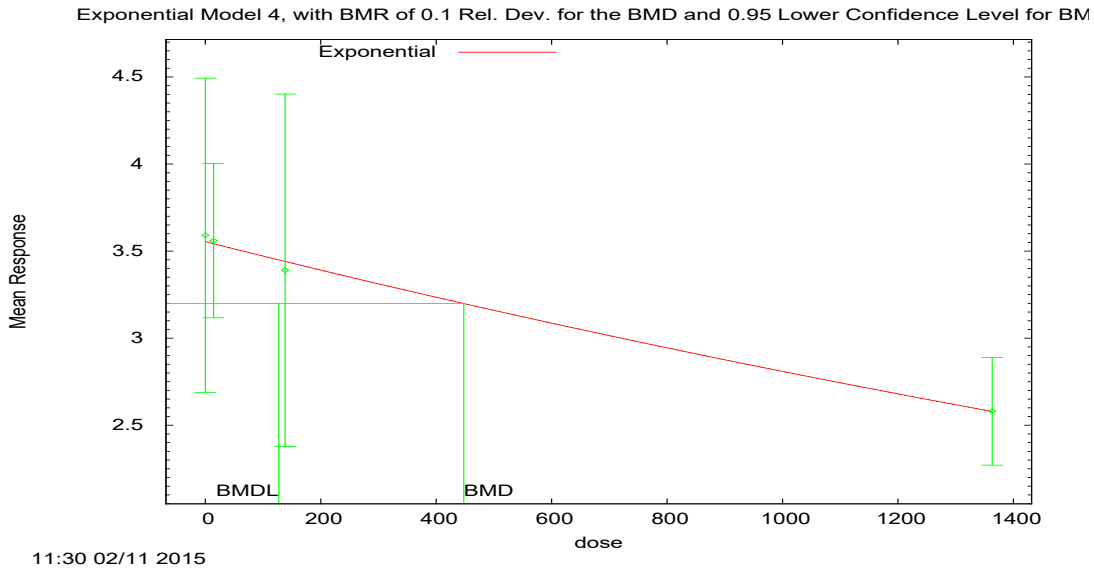
**Tests of Interest**

| 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 |

**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° | 0.424 | 19.323 | 984 | 898 | 1,127 | 1,028 | |
| Polynomial 2° | 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° | 0.424 | 19.323 | 1,240 | 1,132 | 1,360 | 1,193 | |
| Polynomial 2° | 0.414 | 19.368 | 1,181 | 1,030 | 1,357 | 1,115 | |
| Linear | 0.323 | 19.868 | 996 | 757 | 1,344 | 896 | |

[a]Modeled 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.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.




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

**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[dose] = a * [c-(c-1) * exp(-b * 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 |

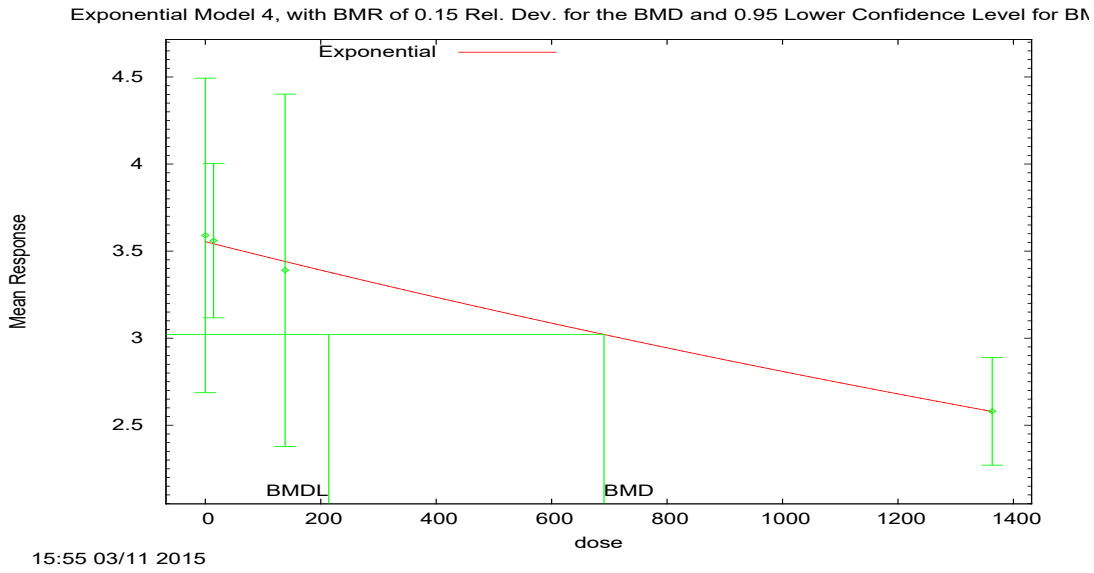
| | | | | | | |
|---|---|---|---|---|---|---|
| 138.3 | 8 | 3.39 | 3.44 | 1.21 | 0.8713 | −0.1637 |
| 1,363 | 8 | 2.58 | 2.579 | 0.37 | 0.3574 | 0.008804 |

**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[dose] = a * [c-(c-1) * exp(-b * 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 |
|---|---|---|
| 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**

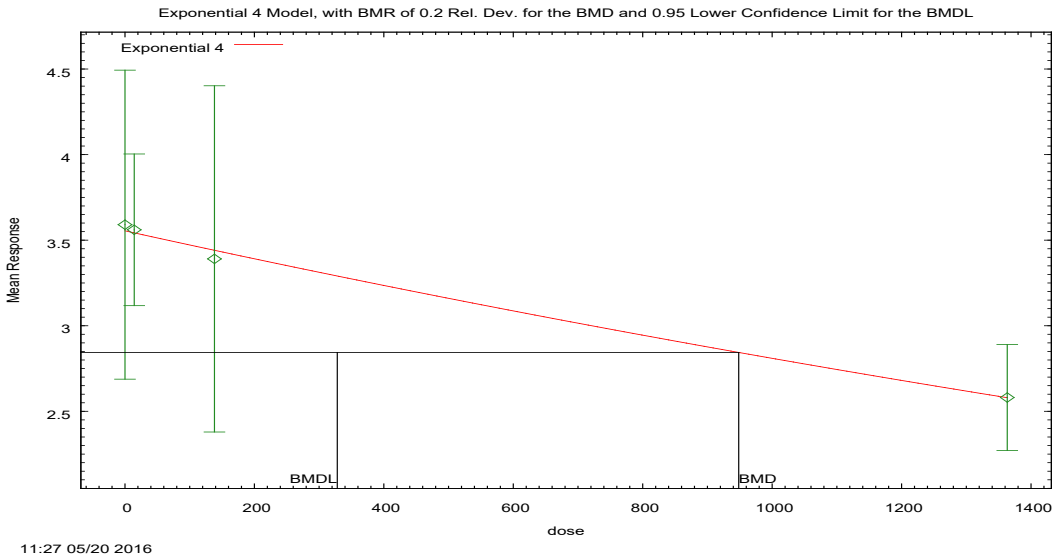
| Dose | N | Observed mean | Estimated mean | Observed SD | Estimated SD | Scaled residuals |
|---|---|---|---|---|---|---|
| 0 | 8 | 3.59 | 3.554 | 1.08 | 0.9635 | 0.105 |
| 14.3 | 8 | 3.56 | 3.542 | 0.53 | 0.9535 | 0.05257 |
| 138.3 | 8 | 3.39 | 3.44 | 1.21 | 0.8713 | −0.1637 |
| 1,363 | 8 | 2.58 | 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 = 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[dose] = a * [c-(c-1) * exp(-b * 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 |

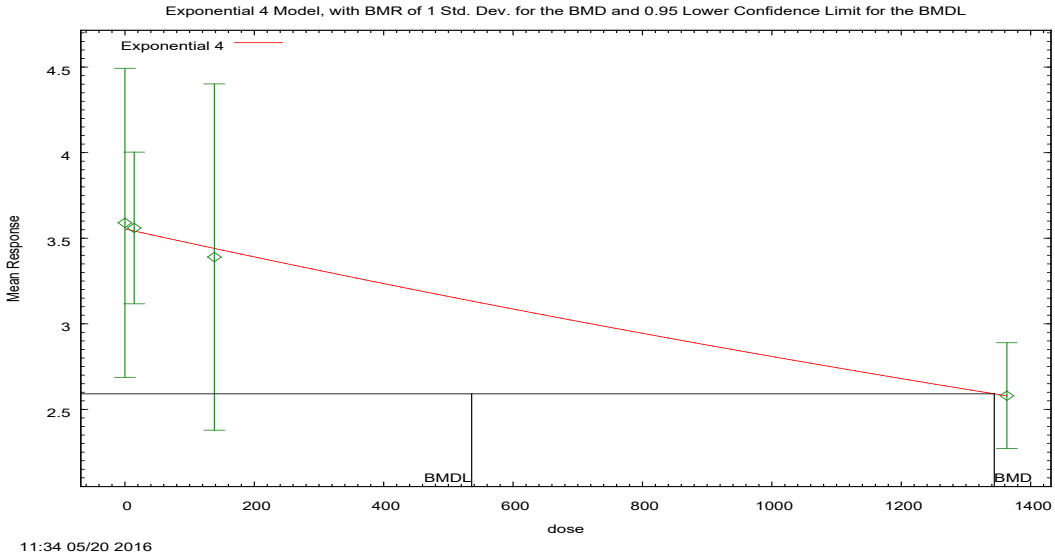
| | | | | | | |
|---|---|---|---|---|---|---|
| 138.3 | 8 | 3.39 | 3.44 | 1.21 | 0.87 | −0.1637 |
| 1,363 | 8 | 2.58 | 2.58 | 0.37 | 0.36 | 0.008804 |

**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[dose] = a * [c-(c-1) * exp(-b * 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 |

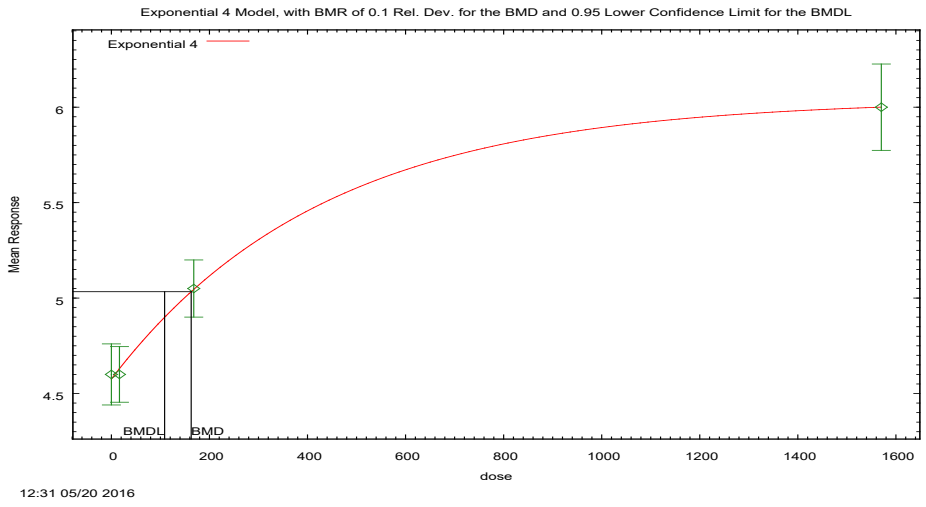
### 3.2.3.2 Liver

**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)<br>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<br>Polynomial 3°[e]<br>Polynomial 2°[f]<br>Linear | 0.00590 | −71.344 | 548 | 480 | 440 | 371 | |

[a]Constant 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.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]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.
[f]For 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 3-13. 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[dose] = a * [c-(c-1) * exp(-b * 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 |

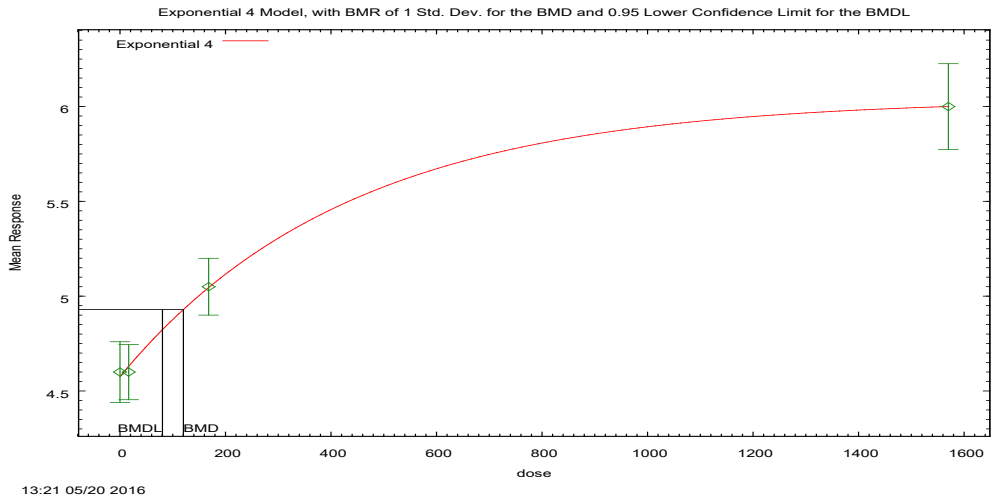
**Likelihoods of Interest**

| 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[dose] = a * [c-(c-1) * exp(-b * 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**

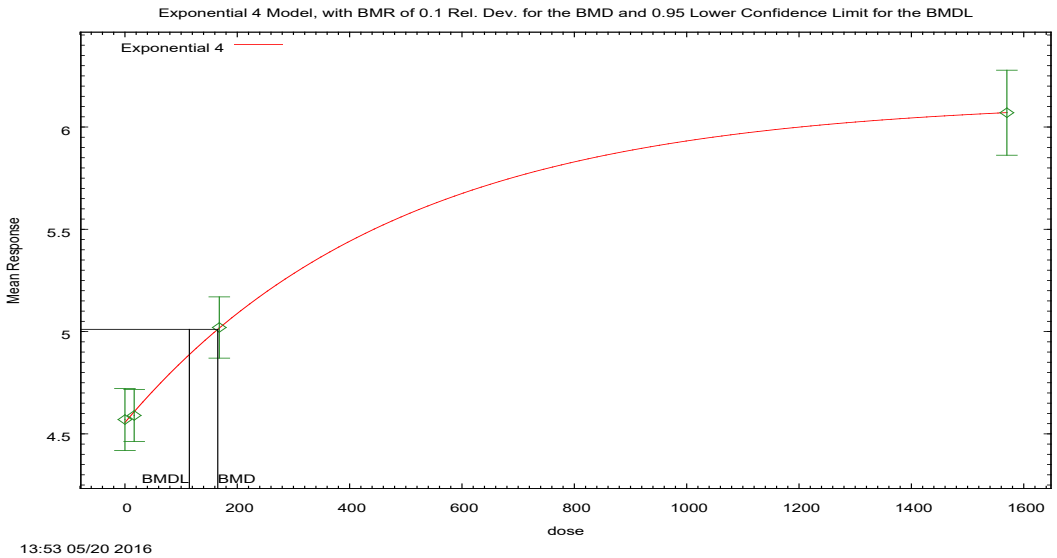
| Test | −2*log (likelihood ratio) | Test df | p-value |
|---|---|---|---|
| Test 1 | 101.3 | 6 | <0.0001 |
| Test 2 | 2.575 | 3 | 0.4619 |
| Test 3 | 2.575 | 3 | 0.4619 |
| Test 6a | 0.2663 | 1 | 0.6058 |

**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)<br>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]<br>Polynomial 3°[e]<br>Polynomial 2°[f]<br>Linear[g] | 0.00403 | −83.646 | 507 | 449 | 371 | 315 | |

[a]Constant 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.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.

[d]The Power model may appear equivalent to the Linear model; however, differences exist in digits not displayed in the table.
[e]For 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.
[f]The Polynomial 2° model may appear equivalent to the Linear model; however, differences exist in digits not displayed in the table.
[g]The 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° and Polynomial 2° models.



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[dose] = a * [c-(c-1) * exp(-b * 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

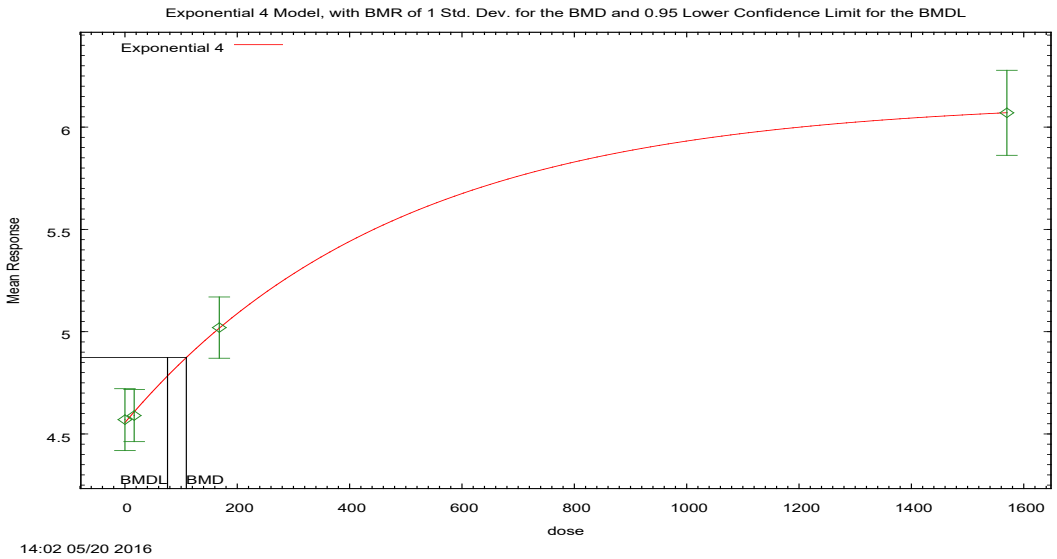
| Dose | N | Observed mean | Estimated mean | Observed SD | Estimated SD | Scaled residuals |
|---|---|---|---|---|---|---|
| 0 | 23 | 4.57 | 4.555 | 0.35 | 0.3184 | 0.2185 |
| 16.5 | 21 | 4.59 | 4.608 | 0.28 | 0.3184 | −0.263 |
| 168 | 20 | 5.02 | 5.017 | 0.32 | 0.3184 | 0.03719 |
| 1,570 | 14 | 6.07 | 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[dose] = a * [c-(c-1) * exp(-b * 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**

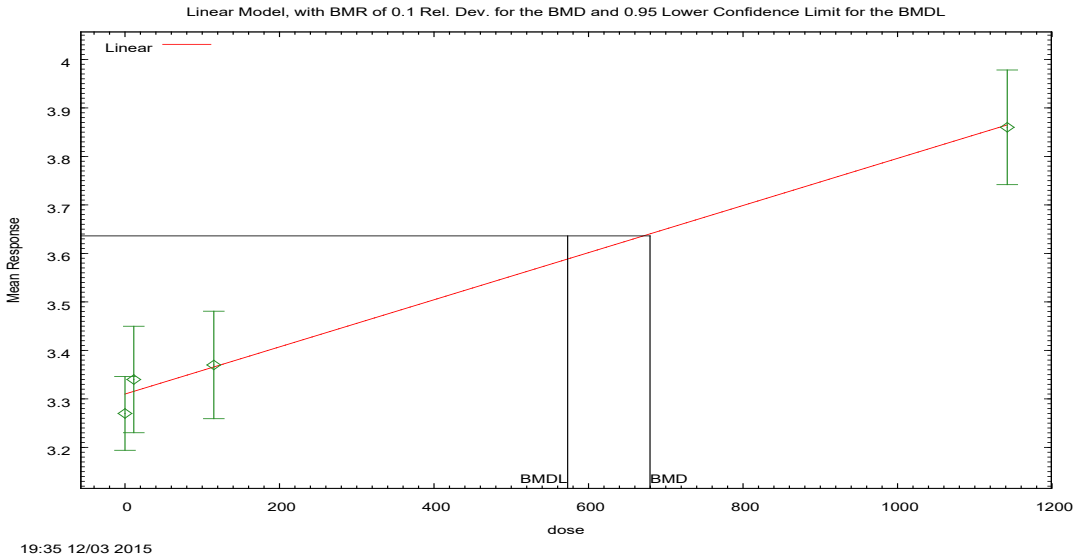
| Test | −2*log (likelihood ratio) | Test df | p-value |
|---|---|---|---|
| Test 1 | 109.5 | 6 | <0.0001 |
| Test 2 | 1.377 | 3 | 0.7109 |
| Test 3 | 1.377 | 3 | 0.7109 |
| Test 6a | 0.1184 | 1 | 0.7308 |

**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)<br>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 models in this case. |
| 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]<br>Polynomial 3°[e]<br>Polynomial 2°[f]<br>Linear | 0.638 | −167.38 | 680 | 573 | 496 | 409 | |

[a]Constant 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.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]BMD or BMDL computation failed for this model.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 coefficient estimate was 0 (boundary of parameters space). he 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.
[f]For 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[dose] = beta_0 + beta_1*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 |

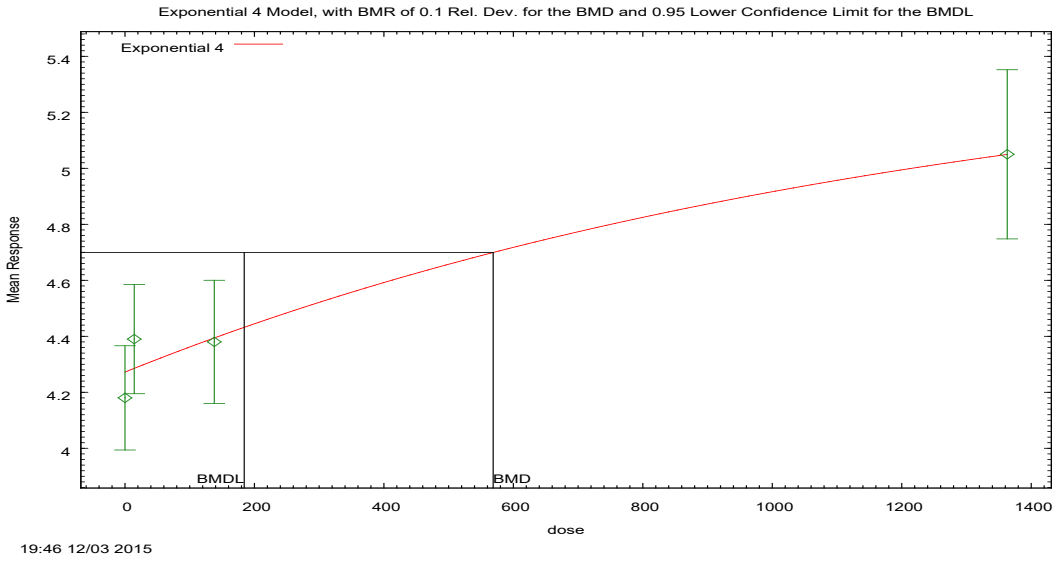
**Table 3-9. Summary of BMD modeling results for relative liver weight (g/100g bw) in F1 adult female CRL Sprague-Dawley rats exposed to HBCD by diet for 17 weeks (Ema et al., 2008); BMR = 10% RD from control mean and 1 SD change from control mean**

| 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)<br>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)<br>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]<br>Polynomial 3°[e]<br>Polynomial 2°[f]<br>Linear | 0.316 | −40.816 | 761 | 578 | 795 | 598 | |

[a]Constant variance case presented (BMDS 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.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]The Exponential (M5) model may appear equivalent to the Exponential (M4) model; however, differences exist in digits not displayed in the table.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]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.
[f]For 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 3-18. 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[dose] = a * [c-(c-1) * exp(-b * 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**

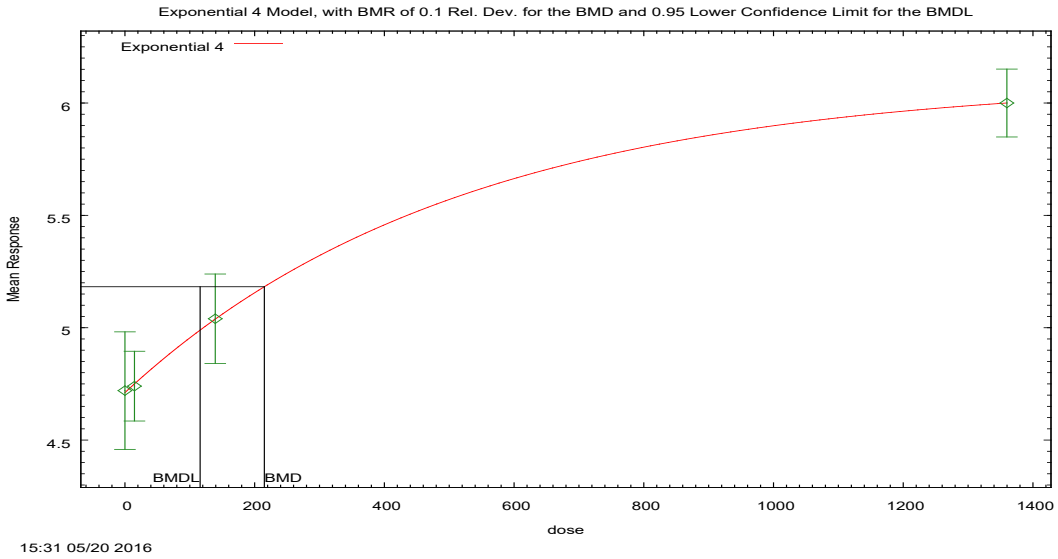
| Test | −2*log (likelihood ratio) | Test df | p-value |
|---|---|---|---|
| Test 1 | 28.1 | 6 | <0.0001 |
| Test 2 | 0.507 | 3 | 0.9174 |
| Test 3 | 0.507 | 3 | 0.9174 |
| Test 6a | 2.188 | 1 | 0.1391 |

**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)<br>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]<br>Polynomial 3°[e]<br>Polynomial 2°[f]<br>Linear | 0.278 | −45.874 | 522 | 438 | 540 | 441 | |

[a]Constant variance case presented. Both constant variance assumption and modeled variance were not appropriate in this case: BMDS Tests 2 and 3 with constatnt 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.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]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.
[f]For 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 3-19. 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[dose] = a * [c-(c-1) * exp(-b * 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 |
|---|---|---|
| Lnalpha | −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 |

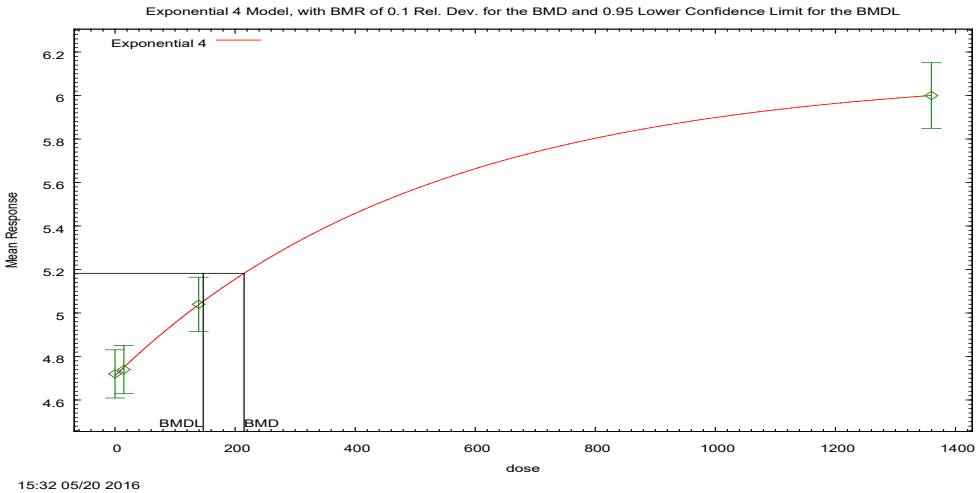
**Sensitivity analysis:**
The fit to the means was adequate for Exponential M4 with constant variance, and their scaled residuals were small. However, Tests 2 and 3 rejected the null hypothesis with both constant variance assumption and modeled variance, indicating lack of fit to variances whether the variance was constant or modeled as a power of the means. To determine how much BMDL10%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)<br>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]<br>Polynomial 3°[e]<br>Polynomial 2°[f]<br>Linear | 0.0241 | −123.60 | 522 | 468 | |

[a]Constant 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.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]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.
[f]For 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 3-20. 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[dose] = a * [c-(c-1) * exp(-b * 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 |
|---|---|---|---|---|---|---|
| 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**

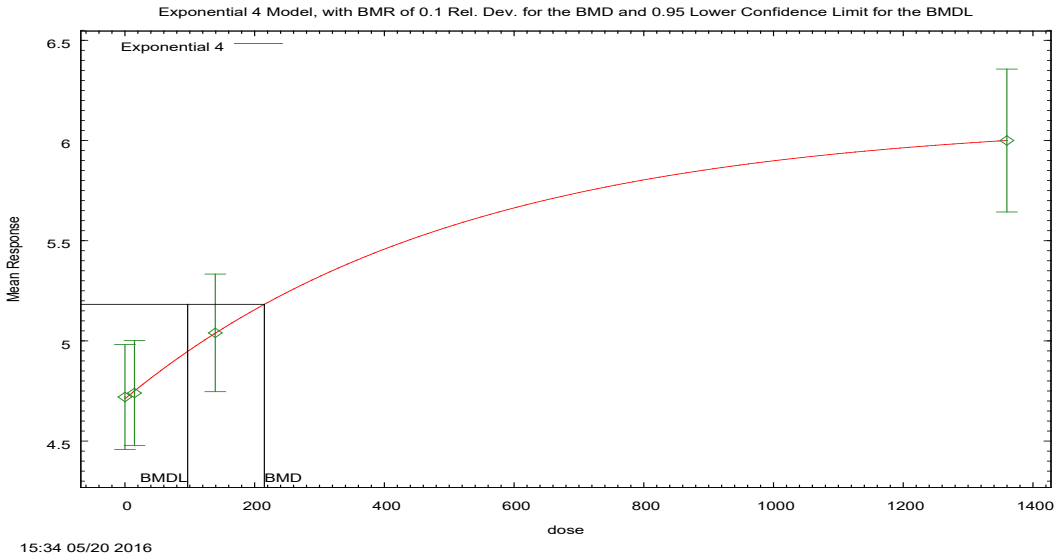
| Test | −2*log (likelihood ratio) | Test df | p-value |
|---|---|---|---|
| Test 1 | 115.3 | 6 | <0.0001 |
| Test 2 | 0.00567 | 3 | 0.9999 |
| Test 3 | 0.00567 | 3 | 0.9999 |
| Test 6a | 0.06685 | 1 | 0.796 |

**Table 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)<br>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]<br>Polynomial 3°[e]<br>Polynomial 2°[f]<br>Linear | 0.498 | −0.86210 | 522 | 414 | |

[a]Constant 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.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]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.
[f]For 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 3-21. 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[dose] = a * [c-(c-1) * exp(-b * 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 |

**Table of Data and Estimated Values of Interest**

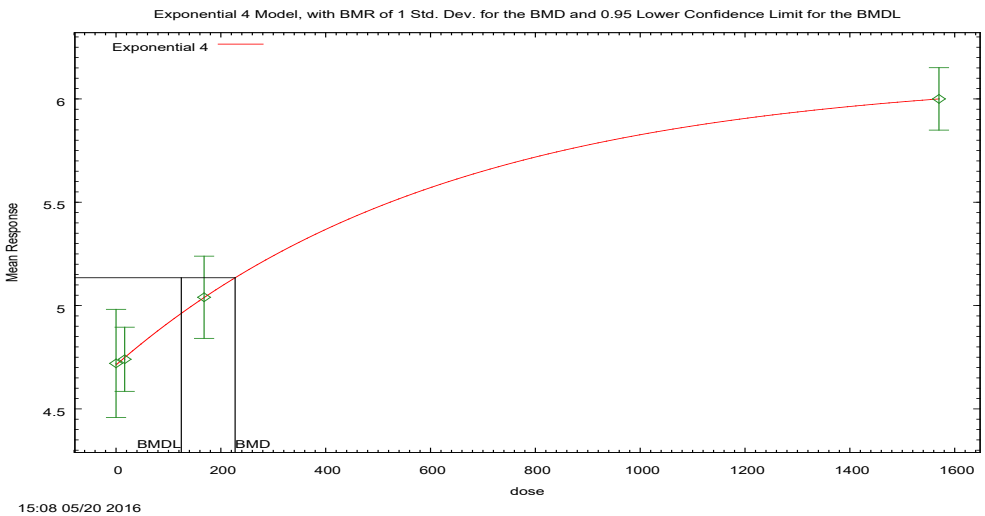
| Dose | N | Observed mean | Estimated mean | Observed SD | Estimated SD | Scaled residuals |
|---|---|---|---|---|---|---|
| 0 | 22 | 4.72 | 4.711 | 0.59 | 0.5741 | 0.07126 |
| 14.7 | 22 | 4.74 | 4.75 | 0.59 | 0.5741 | −0.08227 |
| 139.3 | 18 | 5.04 | 5.038 | 0.59 | 0.5741 | 0.01264 |
| 1,360 | 13 | 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[dose] = a * [c-(c-1) * exp(-b * 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**

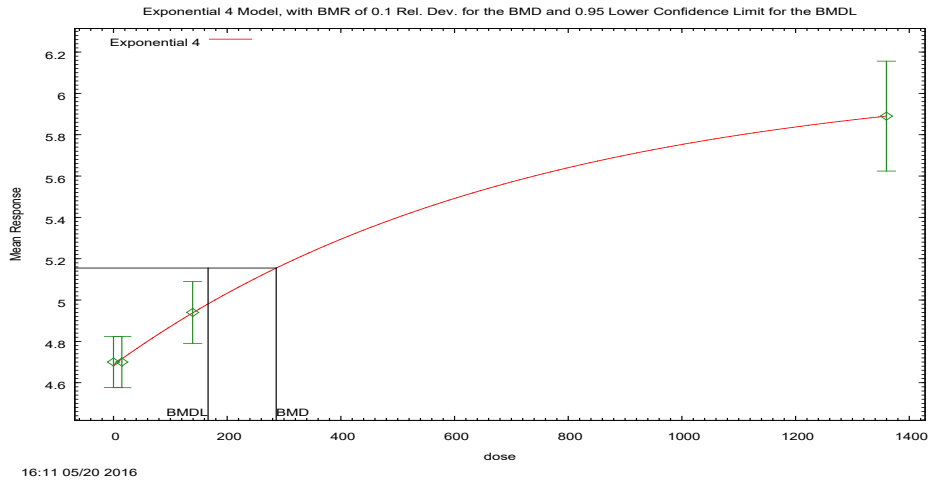
| Test | −2*log (likelihood ratio) | Test df | p-value |
|---|---|---|---|
| Test 1 | 72.69 | 6 | <0.0001 |
| Test 2 | 13.12 | 3 | 0.004382 |
| Test 3 | 13.12 | 3 | 0.004382 |
| Test 6a | 0.016 | 1 | 0.8993 |

**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)<br>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]<br>Polynomial 3°[f]<br>Polynomial 2°[g]<br>Linear | 0.323 | −93.039 | 549 | 477 | 367 | 307 | |

[a]Constant 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.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]BMD or BMDL computation failed for this model.
[e]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[f]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.
[g]For 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 3-23. 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[dose] = a * [c-(c-1) * exp(-b * 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 |
|---|---|---|---|---|---|---|
| 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 |

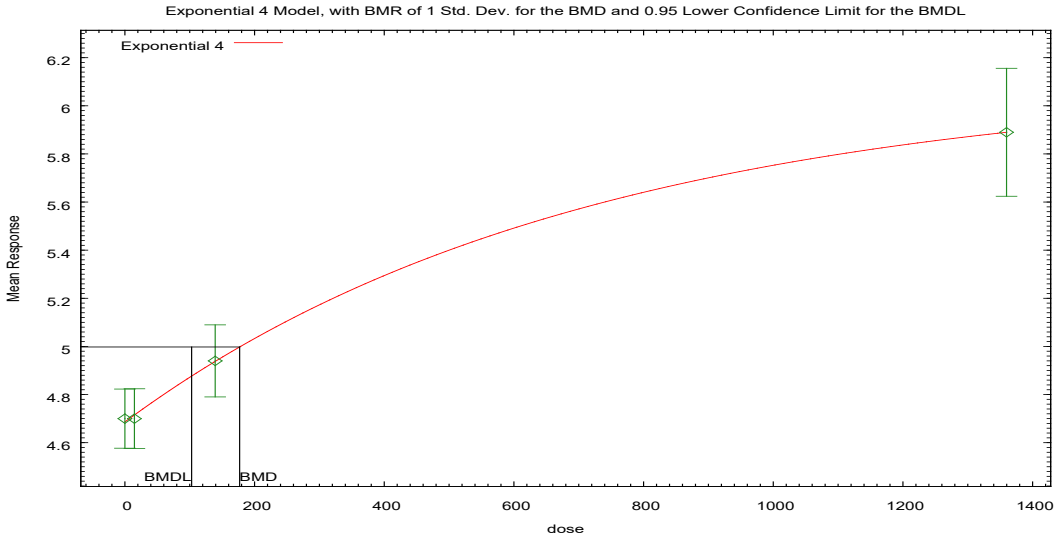
| | | | | | | |
|---|---|---|---|---|---|---|
| 139.3 | 20 | 4.94 | 4.938 | 0.32 | 0.3117 | 0.03152 |
| 1,360 | 13 | 5.89 | 5.89 | 0.44 | 0.3117 | −0.001049 |

**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[dose] = a * [c-(c-1) * exp(-b * 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°[f] Polynomial 2°[g] 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°[f] Polynomial 2°[g] Linear | 0.00161 | −69.324 | 299 | 256 | 282 | 210 | |

[a]Constant 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.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]The Exponential (M4) model may appear equivalent to the Exponential (M5) model; however, differences exist in digits not displayed in the table.
[d]The Exponential (M5) model may appear equivalent to the Exponential (M4) model; however, differences exist in digits not displayed in the table.
[e]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[f]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.
[g]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

**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 | | | | | | | |
| Exponential (M2) Exponential (M3)[b] | <0.0001 | −39.545 | 310 | 261 | 332 | 267 | No model showed adequate fit. Dropping highest dose is not expected to help in this case |
| 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°[e] Polynomial 2°[f] 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 | |
| Powerd Polynomial 3°[e] Polynomial 2°[f] Linear | <0.0001 | −39.727 | 278 | 227 | 327 | 237 | |

[a]Constant 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.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]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.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

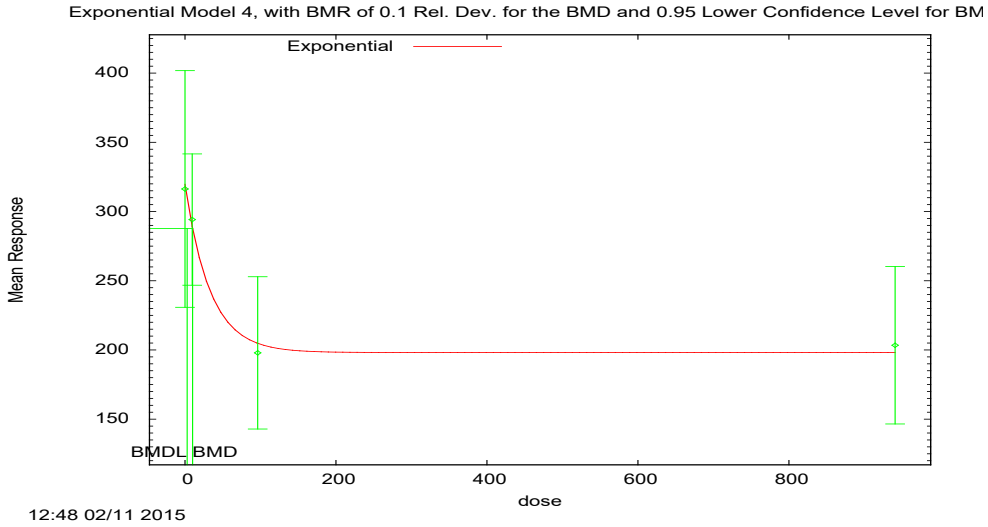
### 3.2.3.3 Reproductive

**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°[f] Linear Polynomial 3°[g] | 0.0117 | 408.78 | 33.1 | 19.8 | 165 | 99.0 | 331 | 198 | |

[a]Constant 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.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]BMD or BMDL computation failed for this model.
[e]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[g]The Polynomial 3° model may appear equivalent to the Linear model; however, differences exist in digits not displayed in the table.

Data from Ema et al. (2008)




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

**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[dose] = a * [c-(c-1) * exp(-b * 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 |

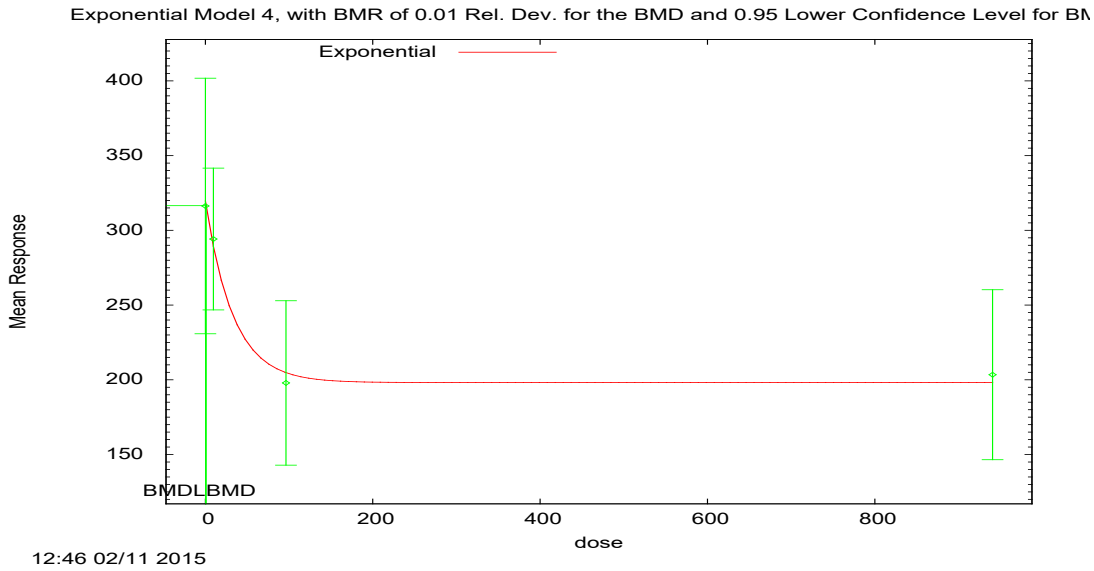
| | | | | | | |
|---|---|---|---|---|---|---|
| 96.3 | 10 | 197.9 | 204.8 | 76.9 | 83.56 | −0.2611 |
| 940.7 | 10 | 203.4 | 198.1 | 79.5 | 83.56 | 0.1987 |

**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 |




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[dose] = a * [c-(c-1) * exp(-b * 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**

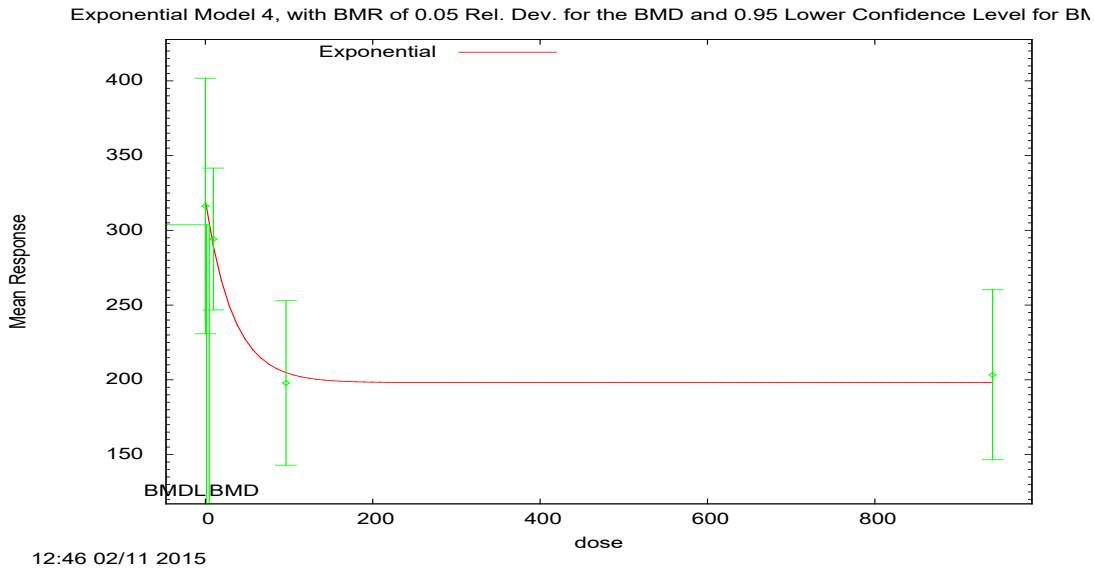
| Dose | N | Observed mean | Estimated mean | Observed SD | Estimated SD | Scaled residuals |
|---|---|---|---|---|---|---|
| 0 | 10 | 316.3 | 319.7 | 119.5 | 83.56 | −0.129 |
| 9.6 | 10 | 294.2 | 289.1 | 66.3 | 83.56 | 0.1915 |
| 96.3 | 10 | 197.9 | 204.8 | 76.9 | 83.56 | −0.2611 |
| 940.7 | 10 | 203.4 | 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[dose] = a * [c-(c-1) * exp(-b * 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 premating 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<br>Weibull<br>Multistage 3°<br>Multistage 2°<br>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] | |

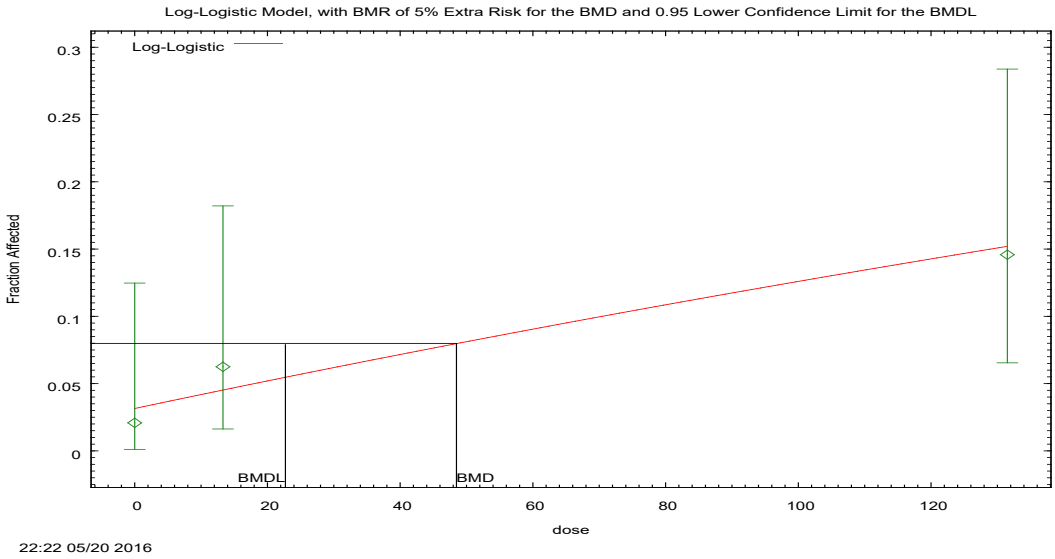
[a]No model was selected as a best-fitting model.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.

**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 premating 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] Quantal-Linear[f] | 0.457 | 76.591 | 51.1 | 25.6 | 105 | 52.5 | |
| Multistage 2°[g] | 0.457 | 76.591 | 51.1 | 25.6 | 105 | 52.5 | |

[a]Selected 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.
[b]The 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° and Quantal-Linear models.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]BMD or BMDL computation failed for this model.
[e]For the Weibull model, the power parameter estimate was 1. The models in this row reduced to the Quantal-Linear model.
[f]The 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° model.
[g]The Multistage 2° 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 premating 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[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(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**

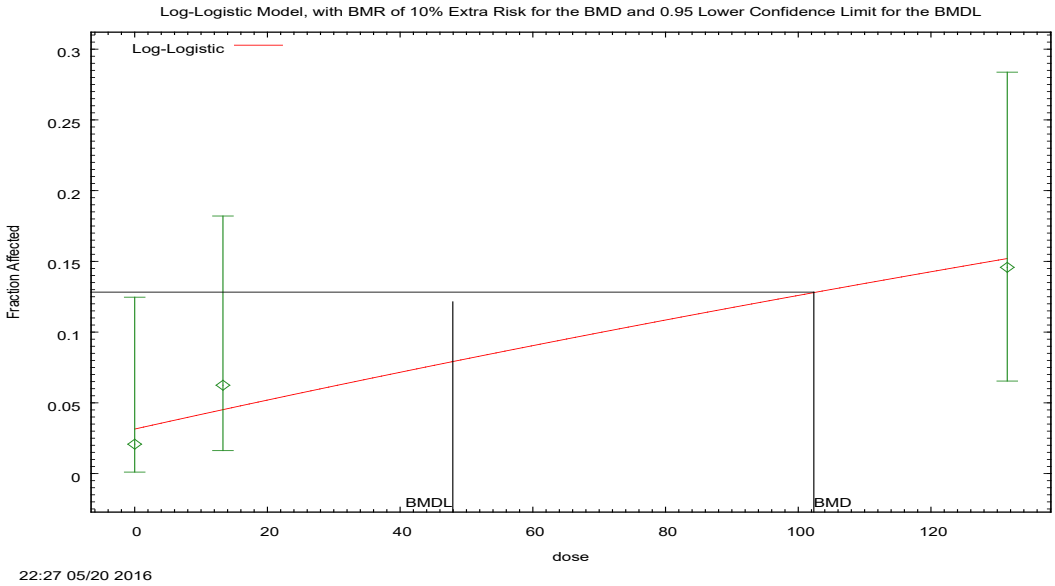
| Model | Log (likelihood) | Number of parameters | Deviance | Test df | p-value |
|---|---|---|---|---|---|
| Full model | −36.0225 | 3 | | | |
| Fitted model | −36.28 | 2 | 0.514904 | 1 | 0.473 |
| Reduced model | −38.8598 | 1 | 5.6746 | 2 | 0.05858 |

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 |

Chi^2 = 0.52, df = 1, p-value = 0.4687




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 premating 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[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(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 |

Chi^2 = 0.52, df = 1, p-value = 0.4687

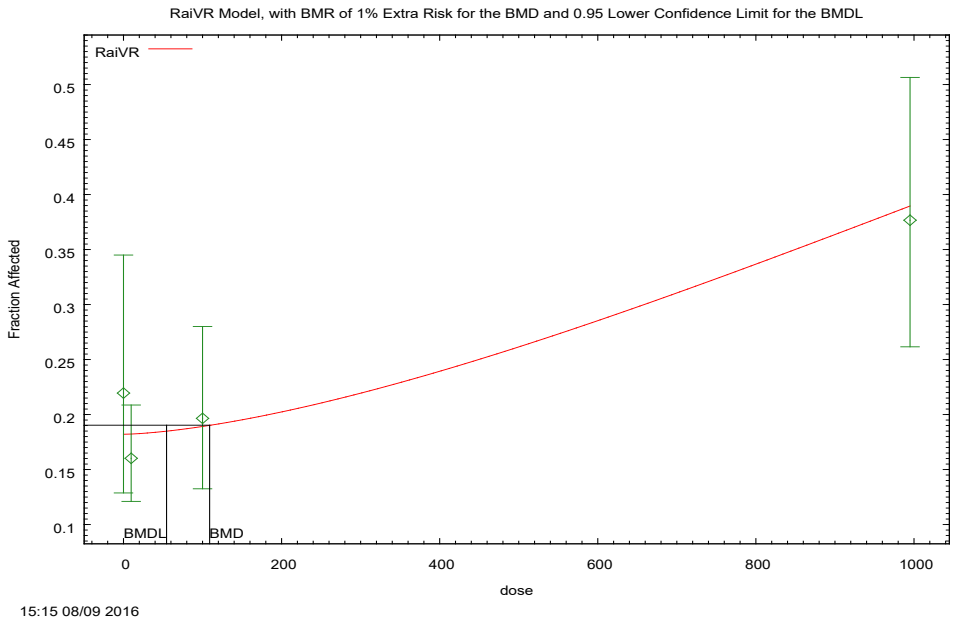
#### 3.2.3.4 Developmental

**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 | |
| NCTRb Rai and Van Ryzin | 0.0000 | 1,336.56 | 136.105 | 68.0523 | 367.95 | 183.975 | |

[a]Because 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.
[b]With 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 3-30. 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:
Prob. = [1-exp(-Alpha-Beta*Dose^Rho)]*exp(-(Th1+Th2*Dose)*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 |

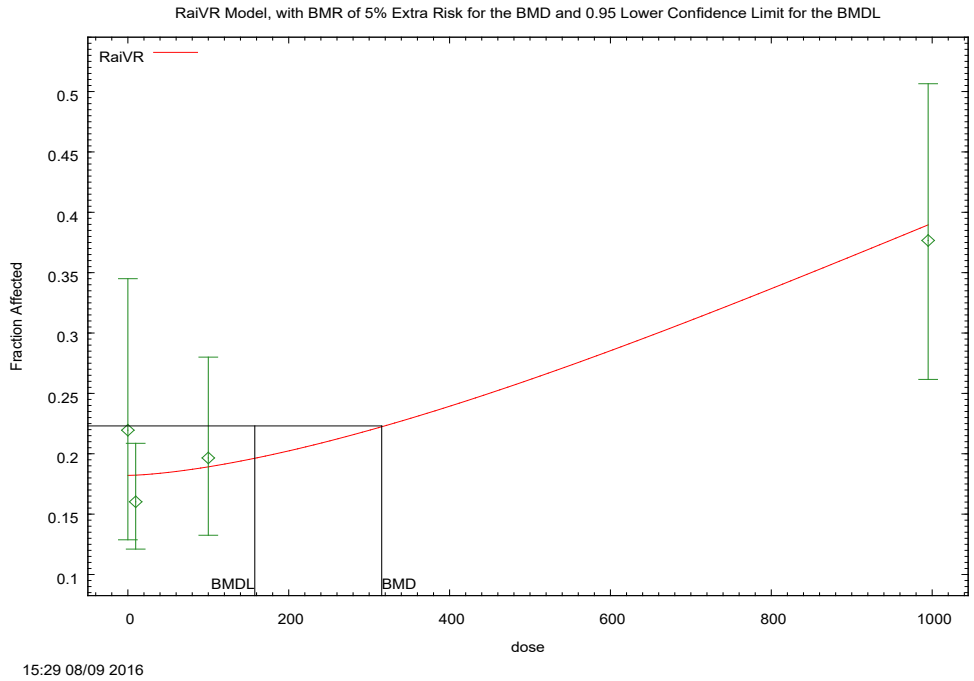
Log-likelihood: −610.162 AIC: 1,234.32

## Goodness-of-Fit Table

| Dose | Lit.-Spec. Cov. | Litter Est._Prob. | Size | Scaled Expected | 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 = 0.1423




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(-Alpha-Beta*Dose^Rho)]*exp(-(Th1+Th2*Dose)*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 | Scaled Expected | 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 |

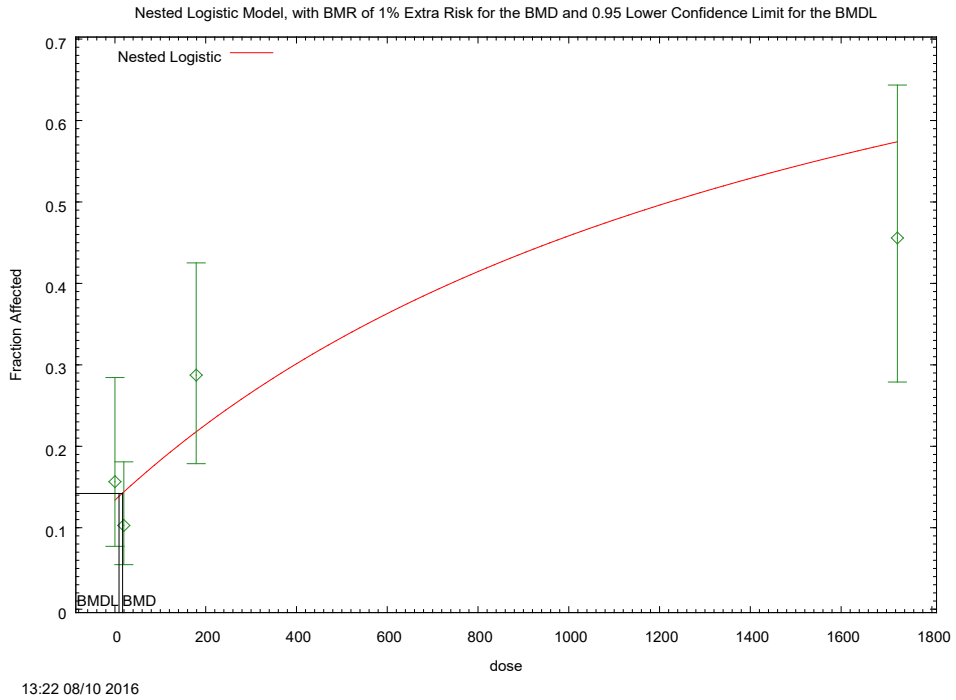
Observed Chi-square = 102.1763 Bootstrap Iterations per run = 10,000
p-value = 0.1416

**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]<br>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]<br>Rai and Van Ryzin | 0.0000 | 656.111 | 35.975 | 17.9875 | 183.603 | 91.8017 | |

[a]Because 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.
[b]With 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:
Prob. = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/
[1+exp(-beta-theta2*Rij-rho*log(Dose))],
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: 14.654762
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 |

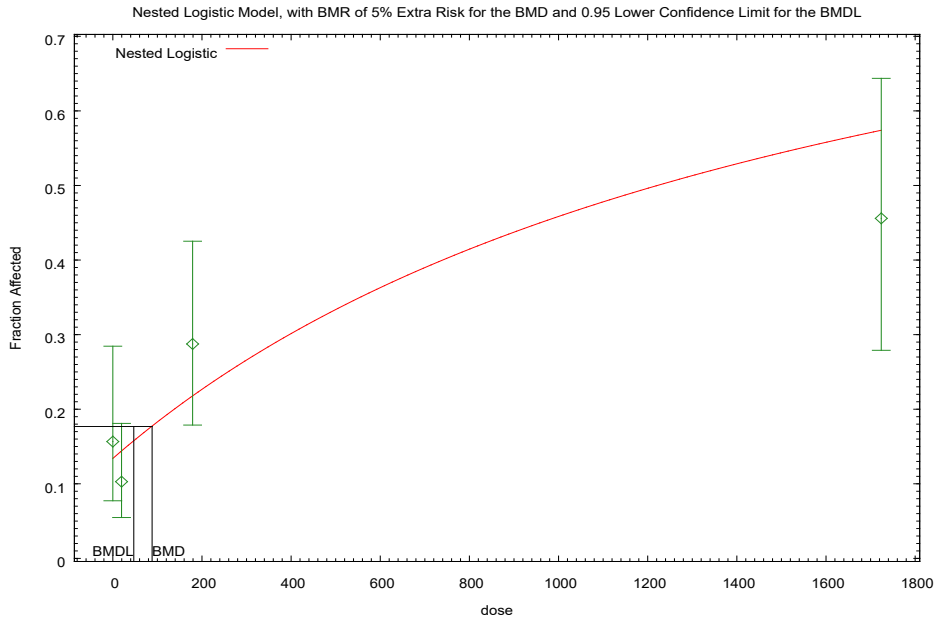
Log-likelihood: −273.736 AIC: 559.472

## Goodness-of-Fit Table

| Dose | Lit.-Spec. Cov. | Litter Est. Prob. | Size | Scaled 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 | 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



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).**

**Nested Logistic Model (Version: 2.20; Date: 04/27/2015)**
The form of the probability function is:
Prob. = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/
[1+exp(-beta-theta2*Rij-rho*log(Dose))],
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: 14.654762
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. Cov. | Litter Est. Prob. | Size | Scaled 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 | 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

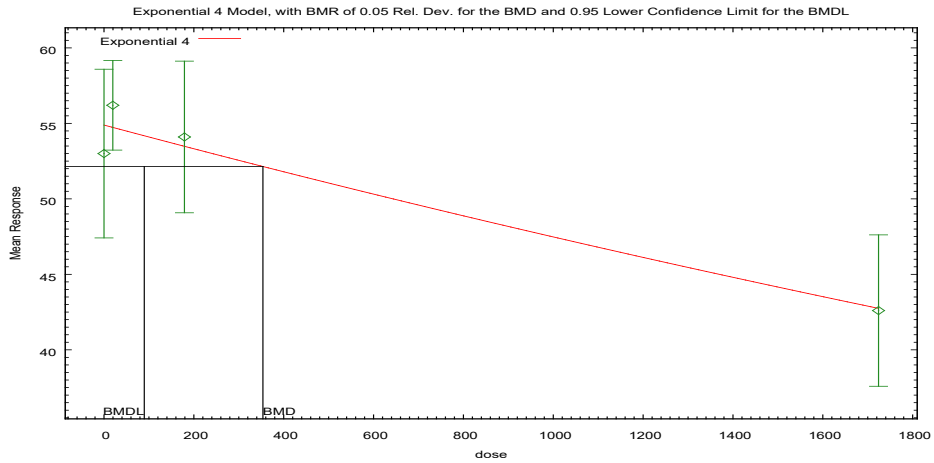
**Table 3-21. Summary of BMD modeling results for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose((Ema et al., 2008); BMR = 5% RD from control mean, 10% RD from control mean, 0.5 SD change from control mean, and 1 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 |
| Exponential (M3) | 0.266 | 422.69 | 651 | 244 | 1016 | 500 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Exponential (M4)** | **0.486** | **420.90** | **354** | **89.6** | 727 | 206 | 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 (M5) | N/A[b] | 424.68 | 230 | 94.0 | 258 | 181 | |
| Hill | N/A[b] | 424.68 | 230 | 89.2 | 264 | errorc | |
| Power | 0.266 | 422.69 | 676 | 282 | 1,049 | 565 | |
| Polynomial 3° Polynomial 2° | 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° Polynomial 2° | 0.264 | 422.70 | 1,091 | 482 | 1,549 | 964 | |
| Linear | 0.497 | 420.85 | 684 | 478 | 1,368 | 956 | |

[a]Constant 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.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD 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[dose] = a * [c-(c-1) * exp(-b * 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**

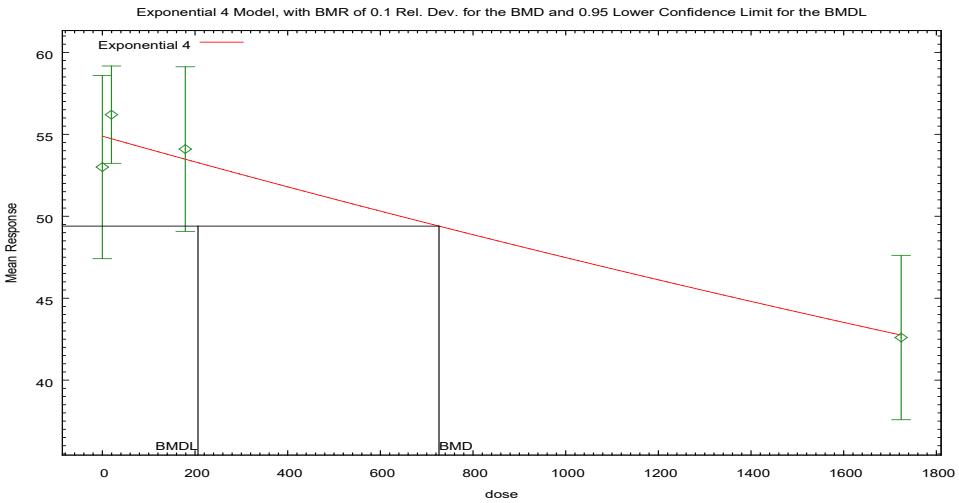
| Dose | N | Observed mean | Estimated mean | Observed SD | Estimated SD | Scaled residuals |
|---|---|---|---|---|---|---|
| 0 | 22 | 53 | 54.89 | 12.6 | 9.64 | −0.9187 |
| 19.6 | 22 | 56.2 | 54.73 | 6.7 | 9.64 | 0.714 |
| 179 | 18 | 54.1 | 53.48 | 10.1 | 9.64 | 0.272 |
| 1,724 | 13 | 42.6 | 42.75 | 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[dose] = a * [c-(c-1) * exp(-b * 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 |

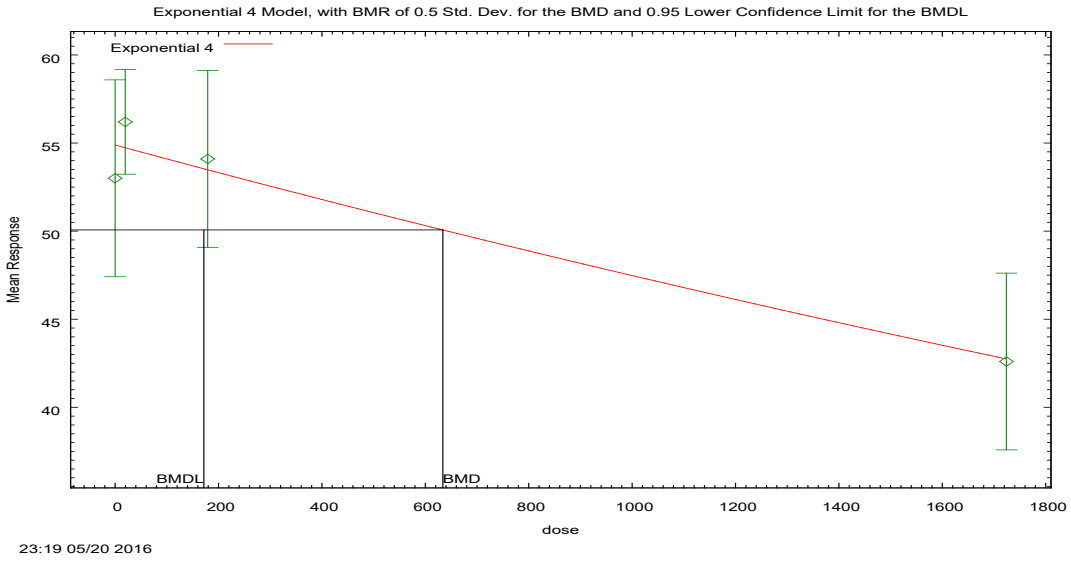
| | | | | | | |
|---|---|---|---|---|---|---|
| 19.6 | 22 | 56.2 | 54.73 | 6.7 | 9.64 | 0.714 |
| 179 | 18 | 54.1 | 53.48 | 10.1 | 9.64 | 0.272 |
| 1,724 | 13 | 42.6 | 42.75 | 8.3 | 9.64 | −0.0551 |

**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[dose] = a * [c-(c-1) * exp(-b * 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**

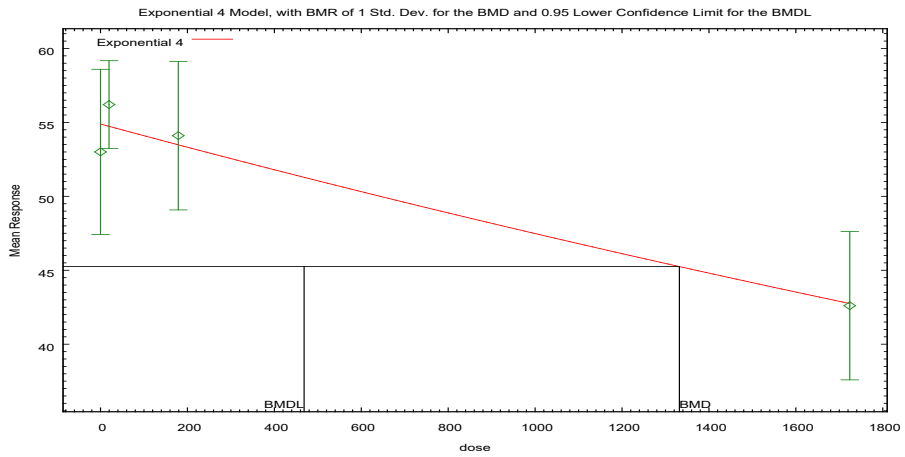
| Dose | N | Observed mean | Estimated mean | Observed SD | Estimated SD | Scaled residuals |
|---|---|---|---|---|---|---|
| 0 | 22 | 53 | 54.89 | 12.6 | 9.64 | −0.9187 |
| 19.6 | 22 | 56.2 | 54.73 | 6.7 | 9.64 | 0.714 |
| 179 | 18 | 54.1 | 53.48 | 10.1 | 9.64 | 0.272 |
| 1,724 | 13 | 42.6 | 42.75 | 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[dose] = a * [c-(c-1) * exp(-b * 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**

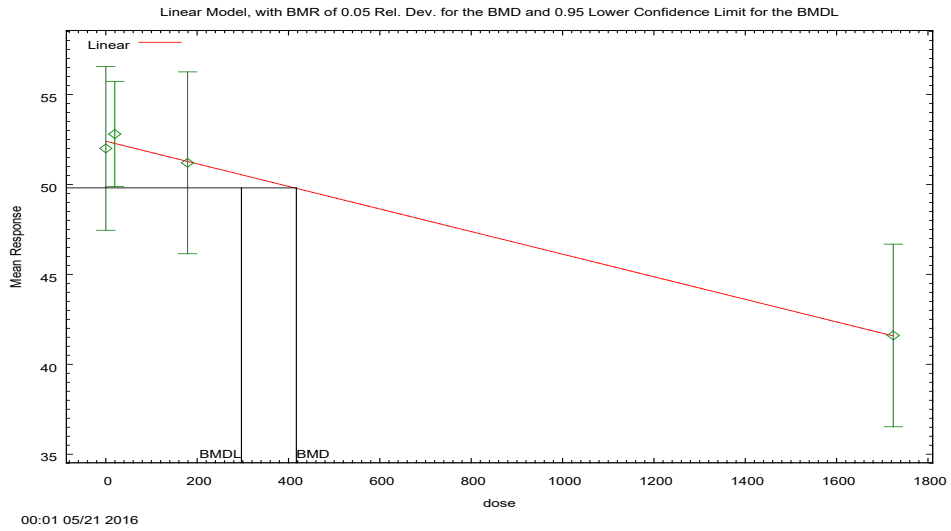
| Test | −2*log (likelihood ratio) | Test df | p-value |
|---|---|---|---|
| Test 1 | 25.12 | 6 | 0.0003244 |
| Test 2 | 9.119 | 3 | 0.02775 |
| Test 3 | 9.119 | 3 | 0.02775 |
| Test 6a | 1.445 | 2 | 0.4856 |

**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°[c] Polynomial 2°[d] 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° Polynomial 2° Linear | 0.942 | 413.8637 | 706 | 489 | 1,412 | 978 | |

[a]Constant 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.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.




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[dose] = beta_0 + beta_1*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**

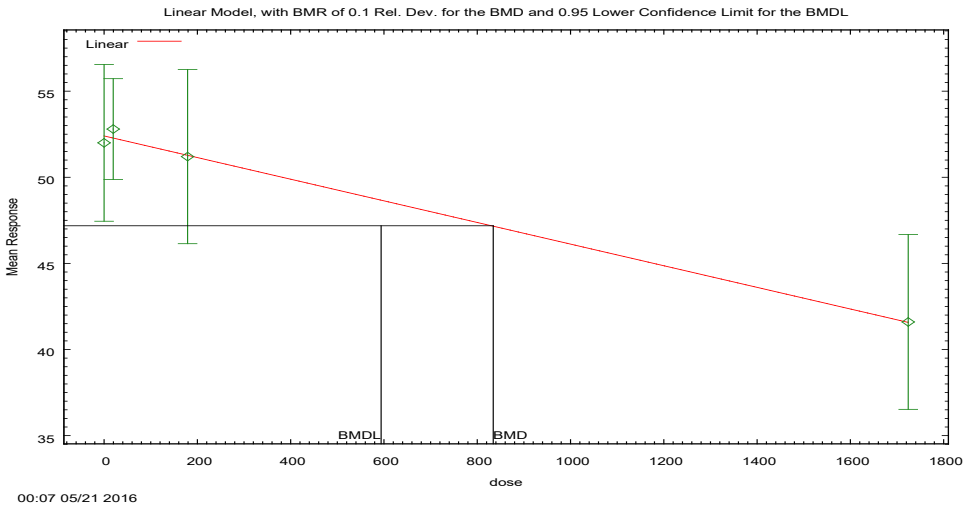
| Dose | N | Observed mean | Estimated mean | Observed SD | Estimated SD | Scaled residuals |
|---|---|---|---|---|---|---|
| 0 | 21 | 52 | 52.4 | 10 | 8.88 | −0.22 |
| 19.6 | 22 | 52.8 | 52.3 | 6.6 | 8.88 | 0.262 |
| 179 | 20 | 51.2 | 51.3 | 10.8 | 8.88 | −0.0514 |
| 1,724 | 13 | 41.6 | 41.6 | 8.4 |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

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 = 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[dose] = beta_0 + beta_1*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**

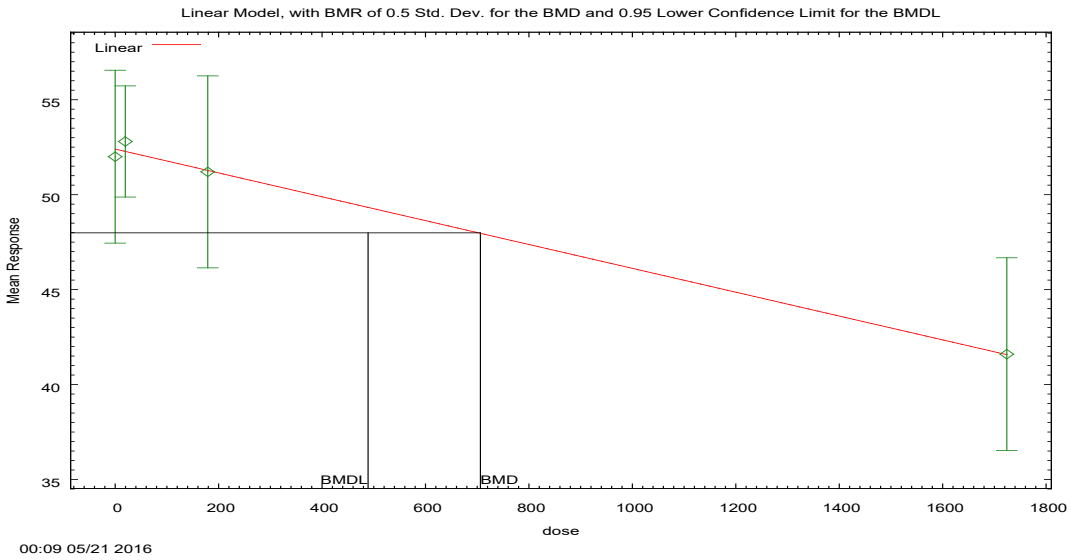
| Dose | N | Observed mean | Estimated mean | Observed SD | Estimated SD | Scaled residuals |
|---|---|---|---|---|---|---|
| 0 | 21 | 52 | 52.4 | 10 | 8.88 | −0.22 |
| 19.6 | 22 | 52.8 | 52.3 | 6.6 | 8.88 | 0.262 |
| 179 | 20 | 51.2 | 51.3 | 10.8 | 8.88 | −0.0514 |
| 1,724 | 13 | 41.6 | 41.6 | 8.4 | 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[dose] = beta_0 + beta_1*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**

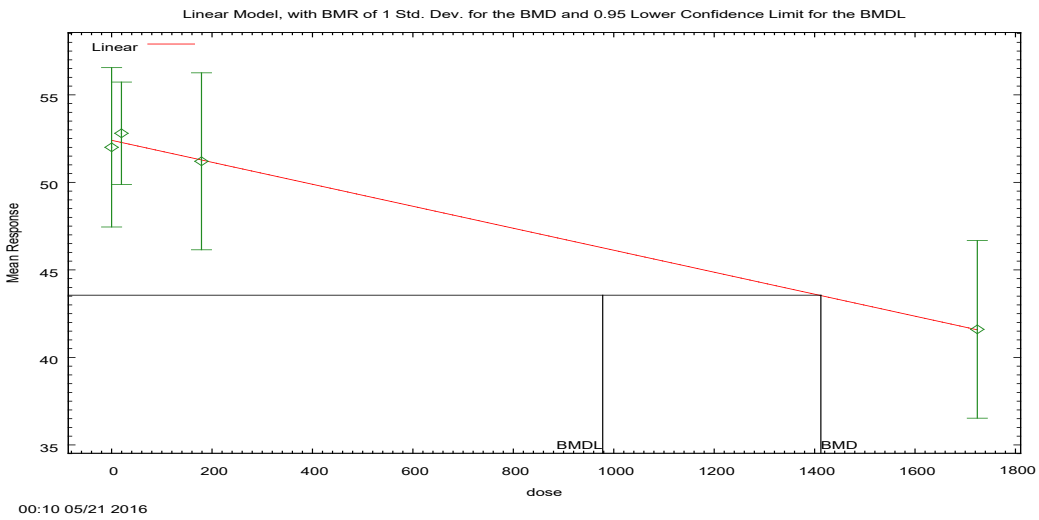
| Dose | N | Observed mean | Estimated mean | Observed SD | Estimated SD | Scaled residuals |
|---|---|---|---|---|---|---|
| 0 | 21 | 52 | 52.4 | 10 | 8.88 | −0.22 |
| 19.6 | 22 | 52.8 | 52.3 | 6.6 | 8.88 | 0.262 |
| 179 | 20 | 51.2 | 51.3 | 10.8 | 8.88 | −0.0514 |
| 1,724 | 13 | 41.6 | 41.6 | 8.4 |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

**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[dose] = beta_0 + beta_1*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 |

# 4 REFERENCES


Al-Mousa, F; Michelangeli, F. (2012). Some commonly used brominated flame retardants cause Ca2+-ATPase inhibition, beta-amyloid peptide release and apoptosis in SH-SY5Y neuronal cells. PLoS ONE 7: e33059. http://dx.doi.org/10.1371/journal.pone.0033059

Al-Mousa, F; Michelangeli, F. (2014). The sarcoplasmic-endoplasmic reticulum Ca2+-ATPase (SERCA) is the likely molecular target for the acute toxicity of the brominated flame retardant hexabromocyclododecane (HBCD). Chem Biol Interact 207: 1-6. http://dx.doi.org/10.1016/j.cbi.2013.10.021

Amacher, DE; Schomaker, SJ; Burkhardt, JE. (1998). The relationship among microsomal enzyme induction, liver weight and histological change in rat toxicology studies. Food Chem Toxicol 36: 831-839. http://dx.doi.org/10.1016/S0278-6915(98)00066-0

Ameribrom. (1990). LETTER FROM AMERIBROM INC TO US EPA REGARDING 8D SUBMISSION FOR HEXABROMOCYCLODODECANE WITH ATTACHMENTS. (TSCATS/410157).

An, J; Zou, W; Chen, C; Zhong, FY; Yu, QZ; Wang, QJ. (2013). The cytological effects of HBCDs on human hepatocyte L02 and the potential molecular mechanism. J Environ Sci Health A Tox Hazard Subst Environ Eng 48: 1333-1342. http://dx.doi.org/10.1080/10934529.2013.781875

An, J; Guo, P; Shang, Y; Zhong, Y; Zhang, X; Yu, Y; Yu, Z. (2016). The "adaptive responses" of low concentrations of HBCD in L02 cells and the underlying molecular mechanisms. Chemosphere 145: 68-76. http://dx.doi.org/10.1016/j.chemosphere.2015.11.071.

Andersen, S; Pedersen, KM; Bruun, NH; Laurberg, P. (2002). Narrow individual variations in serum T4 and T3 in normal subjects: a clue to the understanding of subclinical thyroid disease. J Clin Endocrinol Metab 87: 1068-1072. http://dx.doi.org/10.1210/jcem.87.3.8165

Ausó, E; Lavado-Autric, R; Cuevas, E; Del Rey, FE; Morreale De Escobar, G; Berbel, P. (2004). A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology 145: 4037-4047. http://dx.doi.org/10.1210/en.2004-0274

Bailey, SA; Zidell, RH; Perry, RW. (2004). Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint? Toxicol Pathol 32: 448-466. http://dx.doi.org/10.1080/01926230490465874

Basf. (2000). Cytogenetic study in vivo with of hexabromocyclododecane in the mouse micronucleus test after two intraperitoneal administrations. (Project No. 26MO100/004018). Ludwigshafen, Germany: BASF Aktiengesellschaft.

Bernhard, A; Berntssen, MH; Lundebye, AK; Røyneberg Alvheim, A; Secher Myrmel, L; Fjære, E; Torstensen, BE; Kristiansen, K; Madsen, L; Brattelid, T; Rasinger, JD. (2016). Marine fatty acids aggravate hepatotoxicity of α-HBCD in juvenile female BALB/c mice. Food Chem Toxicol 97: 411-423. http://dx.doi.org/10.1016/j.fct.2016.10.002

Boone, L; Meyer, D; Cusick, P; Ennulat, D; Bolliger, AP; Everds, N; Meador, V; Elliott, G; Honor, D; Bounous, D; Jordan, H. (2005). Selection and interpretation of clinical

pathology indicators of hepatic injury in preclinical studies [Review]. Vet Clin Pathol 34: 182-188. http://dx.doi.org/10.1111/j.1939-165X.2005.tb00041.x
Burns-Naas, LA; Hastings, KL; Ladics, GS; Makris, SL; Parker, GA; Holsapple, MP. (2008). What's so special about the developing immune system? [Review]. Int J Toxicol 27: 223-254. http://dx.doi.org/10.1080/10915810801978110
Cantón, RF; Peijnenburg, AA; Hoogenboom, RL; Piersma, AH; van der Ven, LT; van den Berg, M; Heneweer, M. (2008). Subacute effects of hexabromocyclododecane (HBCD) on hepatic gene expression profiles in rats. Toxicol Appl Pharmacol 231: 267-272. http://dx.doi.org/10.1016/j.taap.2008.04.013
Cantón, RF; Sanderson, JT; Nijmeijer, S; Bergman, A; Letcher, RJ; van den Berg, M. (2006). In vitro effects of brominated flame retardants and metabolites on CYP17 catalytic activity: a novel mechanism of action? Toxicol Appl Pharmacol 216: 274-281. http://dx.doi.org/10.1016/j.taap.2006.05.007
Chen, C; Staudinger, JL; Klaassen, CD. (2003). Nuclear receptor, pregname X receptor, is required for induction of UDP-glucuronosyltranferases in mouse liver by pregnenolone-16 alpha-carbonitrile. Drug Metab Dispos 31: 908-915. http://dx.doi.org/10.1124/dmd.31.7.908
Christen, V; Crettaz, P; Oberli-Schrämmli, A; Fent, K. (2010). Some flame retardants and the antimicrobials triclosan and triclocarban enhance the androgenic activity in vitro. Chemosphere 81: 1245-1252. http://dx.doi.org/10.1016/j.chemosphere.2010.09.031
Crump, D; Chiu, S; Egloff, C; Kennedy, SW. (2008). Effects of hexabromocyclododecane and polybrominated diphenyl ethers on mRNA expression in chicken (Gallus domesticus) hepatocytes. Toxicol Sci 106: 479-487. http://dx.doi.org/10.1093/toxsci/kfn196
Crump, D; Egloff, C; Chiu, S; Letcher, RJ; Chu, S; Kennedy, SW. (2010). Pipping success, isomer-specific accumulation, and hepatic mRNA expression in chicken embryos exposed to HBCD. Toxicol Sci 115: 492-500. http://dx.doi.org/10.1093/toxsci/kfq068
Deng, J; Yu, L; Liu, C; Yu, K; Shi, X; Yeung, LW; Lam, PK; Wu, RS; Zhou, B. (2009). Hexabromocyclododecane-induced developmental toxicity and apoptosis in zebrafish embryos. Aquat Toxicol 93: 29-36. http://dx.doi.org/10.1016/j.aquatox.2009.03.001
Dingemans, MM; Heusinkveld, HJ; de Groot, A; Bergman, A; van den Berg, M; Westerink, RH. (2009). Hexabromocyclododecane inhibits depolarization-induced increase in intracellular calcium levels and neurotransmitter release in PC12 cells. Toxicol Sci 107: 490-497. http://dx.doi.org/10.1093/toxsci/kfn249
Dorosh, A; Děd, L; Elzeinová, F; Pěknicová, J. (2011). Assessing oestrogenic effects of brominated flame retardants hexabromocyclododecane and tetrabromobisphenol A on MCF-7 cells. Folia Biol (Krakow Pol) 57: 35-39.
Du, M; Zhang, D; Yan, C; Zhang, X. (2012). Developmental toxicity evaluation of three hexabromocyclododecane diastereoisomers on zebrafish embryos. Aquat Toxicol 112-113: 1-10. http://dx.doi.org/10.1016/j.aquatox.2012.01.013
Eggesbø, M; Thomsen, C; Jørgensen, JV; Becher, G; Odland, JØ; Longnecker, MP. (2011). Associations between brominated flame retardants in human milk and thyroid-stimulating hormone (TSH) in neonates. Environ Res 111: 737-743. http://dx.doi.org/10.1016/j.envres.2011.05.004
Ema, M; Fujii, S; Hirata-Koizumi, M; Matsumoto, M. (2008). Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats. Reprod Toxicol 25: 335-351. http://dx.doi.org/10.1016/j.reprotox.2007.12.004

Emea. (2008). Non-clinical guideline on drug induced hepatotoxicity. (Doc. Ref. EMEA/CHMP/SWP/150115/2006). London, UK. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003355.pdf

Eriksson, P; Fischer, C; Wallin, M; Jakobsson, E; Fredriksson, A. (2006). Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD). Environ Toxicol Pharmacol 21: 317-322. http://dx.doi.org/10.1016/j.etap.2005.10.001

Eshraghian, A; Jahromi, AH. (2014). Non-alcoholic fatty liver disease and thyroid dysfunction: A systematic review. World J Gastroenterol 20: 8102–8109. http://dx.doi.org/10.3748/wjg.v20.i25.8102

Ethyl, C. (1990a). Genetic toxicology rat hepatocyte primary culture/DNA repair test on hexabromocyclododecane with cover letter dated 030890. (TSCATS/405817. OTS0522234. Doc I.D. 86900000163). Baton Rouge, LA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0522234

Ethyl, C. (1990b). Genetic toxicology salmonella/microsomal assay on hexabromocyclododecane with cover letter dated 030890 [TSCA Submission]. (TSCATS/405818. OTS0522235. Doc I.D. 86900000164). Baton Rouge, LA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0522235

Fa, S; Pogrmic-Majkic, K; Dakic, V; Kaisarevic, S; Hrubik, J; Andric, N; Stojilkovic, SS; Kovacevic, R. (2013). Acute effects of hexabromocyclododecane on Leydig cell cyclic nucleotide signaling and steroidogenesis in vitro. Toxicol Lett 218: 81-90. http://dx.doi.org/10.1016/j.toxlet.2013.01.009

Fa, S; Pogrmic-Majkic, K; Samardzija, D; Hrubik, J; Glisic, B; Kovacevic, R; Andric, N. (2015). HBCDD-induced sustained reduction in mitochondrial membrane potential, ATP and steroidogenesis in peripubertal rat Leydig cells. Toxicol Appl Pharmacol 282: 20-29. http://dx.doi.org/10.1016/j.taap.2014.11.001

Fa, S; Samardzija, D; Odzic, L; Pogrmic-Majkic, K; Kaisarevic, S; Kovacevic, R; Andric, N. (2014). Hexabromocyclododecane facilitates FSH activation of ERK1/2 and AKT through epidermal growth factor receptor in rat granulosa cells. Arch Toxicol 88: 345-354. http://dx.doi.org/10.1007/s00204-013-1133-2

Fernandez Canton, R; Sanderson, T; Nijmeijer, S; Bergman, A; Van Den Berg, M. (2005). In vitro effects of brominated flame retardants on the adrenocortical enzyme CYP17. A novel endocrine mechanism of action? [Abstract]. Toxicologist 84: 356.

Finken, MJ; van Eijsden, M; Loomans, EM; Vrijkotte, TG; Rotteveel, J. (2013). Maternal hypothyroxinemia in early pregnancy predicts reduced performance in reaction time tests in 5- to 6-year-old offspring. J Clin Endocrinol Metab 98: 1417-1426. http://dx.doi.org/10.1210/jc.2012-3389

Fisher, DA; Nelson, JC. (2012). Application of TSH and free thyroxine measurements to thyroid diagnosis: Laboratory support of diagnosis and management. Fisher, DA; Nelson, JC. http://www.questdiagnostics.com/testcenter/testguide.action?dc=WP_AppTSH

Forhead, AJ; Fowden, AL. (2014). Thyroid hormones in fetal growth and prepartum maturation [Review]. J Endocrinol 221: R87-R103. http://dx.doi.org/10.1530/JOE-14-0025

Fujimoto, H; Woo, GH; Morita, R; Itahashi, M; Akane, H; Nishikawa, A; Shibutani, M. (2013). Increased cellular distribution of vimentin and ret in the cingulum of rat offspring after developmental exposure to decabromodiphenyl ether or 1,2,5,6,9,10-

hexabromocyclododecane. J Toxicol Pathol 26: 119-129. http://dx.doi.org/10.1293/tox.26.119

Fujimoto, H; Woo, Gy; Inoue, K; Igarashi, K; Kanno, Ju; Hirose, M; Nishikawa, A; Shibutani, M. (2012). Increased cellular distribution of vimentin and Ret in the cingulum induced by developmental hypothyroidism in rat offspring maternally exposed to anti-thyroid agents. Reprod Toxicol 34: 93-100. http://dx.doi.org/10.1016/j.reprotox.2012.03.005

Furlow, JD; Yang, HY; Hsu, M; Lim, W; Ermio, DJ; Chiellini, G; Scanlan, TS. (2004). Induction of larval tissue resorption in Xenopus laevis tadpoles by the thyroid hormone receptor agonist GC-1. J Biol Chem 279: 26555-26562. http://dx.doi.org/10.1074/jbc.M402847200

Genskow, KR; Bradner, JM; Hossain, MM; Richardson, JR; Caudle, WM. (2015). Selective damage to dopaminergic transporters following exposure to the brominated flame retardant, HBCDD. Neurotoxicol Teratol 52: 162-169. http://dx.doi.org/10.1016/j.ntt.2015.06.003

Germer, S; Piersma, AH; van der Ven, L; Kamyschnikow, A; Fery, Y; Schmitz, HJ; Schrenk, D. (2006). Subacute effects of the brominated flame retardants hexabromocyclododecane and tetrabromobisphenol A on hepatic cytochrome P450 levels in rats. Toxicology 218: 229-236. http://dx.doi.org/10.1016/j.tox.2005.10.019

Gilbert, ME. (2011). Impact of low-level thyroid hormone disruption induced by propylthiouracil on brain development and function. Toxicol Sci 124: 432-445. http://dx.doi.org/10.1093/toxsci/kfr244

Gilbert, ME; Hedge, JM; Valentin-Blasini, L; Blount, BC; Kannan, K; Tietge, J; Zoeller, RT; Crofton, KM; Jarrett, JM; Fisher, JW. (2013). An animal model of marginal iodine deficiency during development: The thyroid axis and neurodevelopmental outcome. Toxicol Sci 132: 177-195. http://dx.doi.org/10.1093/toxsci/kfs335

Gilbert, ME; Ramos, RL; McCloskey, DP; Goodman, JH. (2014). Subcortical band heterotopia in rat offspring following maternal hypothyroxinaemia: structural and functional characteristics. J Neuroendocrinol 26: 528-541. http://dx.doi.org/10.1111/jne.12169

Gilbert, ME; Rovet, J; Chen, Z; Koibuchi, N. (2012). Developmental thyroid hormone disruption: prevalence, environmental contaminants and neurodevelopmental consequences. Neurotoxicology 33: 842-852. http://dx.doi.org/10.1016/j.neuro.2011.11.005

Gilbert, ME; Sanchez-Huerta, K; Wood, C. (2016). Mild thyroid hormone insufficiency during development compromises activity-dependent neuroplasticity in the hippocampus of adult male rats. Endocrinology 157: 774-787. http://dx.doi.org/10.1210/en.2015-1643

Gilbert, ME; Zoeller, RT. (2010). Thyroid hormones--impact on the developing brain: Possible mechanisms of neurotoxicity. In GJ Harry; HA Tilson (Eds.), Target Organ Toxicology Series, vol 28 (3rd ed., pp. 79-111). New York, NY: Informa Healthcare.

Gsri. (1978). Mutagenicity test of GLS-S6-41A (not published). (TSCATS/443581. OTS0000947. EPA/OTS Doc #FYI-OTS-0794-0947).

Hachisuka, A; Nakamura, R; Sato, Y; Nakamura, R; Shibutani, M; Teshima, R. (2010). Effects of perinatal exposure to the brominated flame-retardant hexabromocyclododecane (HBCD) on the developing immune system in rats [translation]. Kokuritsu Iyakuhin Shokuhin Eisei Kenkyūjo Hōkoku (128): 58-64.

Haddow, JE; Palomaki, GE; Allan, WC; Williams, JR; Knight, GJ; Gagnon, J; O'Heir, CE; Mitchell, ML; Hermos, RJ; Waisbren, SE; Faix, JD; Klein, RZ. (1999). Maternal thyroid

deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 341: 549-555. http://dx.doi.org/10.1056/NEJM199908193410801

Hall, AP; Elcombe, CR; Foster, JR; Harada, T; Kaufmann, W; Knippel, A; Küttler, K; Malarkey, DE; Maronpot, RR; Nishikawa, A; Nolte, T; Schulte, A; Strauss, V; York, MJ. (2012). Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop [Review]. Toxicol Pathol 40: 971-994. http://dx.doi.org/10.1177/0192623312448935

Hamers, T; Kamstra, JH; Sonneveld, E; Murk, AJ; Kester, MH; Andersson, PL; Legler, J; Brouwer, A. (2006). In vitro profiling of the endocrine-disrupting potency of brominated flame retardants. Toxicol Sci 92: 157-173. http://dx.doi.org/10.1093/toxsci/kfj187

Heindel, JJ. (1998). An evaluation and interpretation of reproductive endpoints for human health risk assessment: Oocyte quantitation and ovarian histology. Washington, DC: ILSI Press.

Helleday, T; Tuominen, KL; Bergman, A; Jenssen, D. (1999). Brominated flame retardants induce intragenic recombination in mammalian cells. Mutat Res 439: 137-147. http://dx.doi.org/10.1016/S1383-5718(98)00186-7

Henics, T; Wheatley, DN. (1999). Cytoplasmic vacuolation, adaptation and cell death: a view on new perspectives and features [Review]. Biol Cell 91: 485-498. http://dx.doi.org/10.1016/S0248-4900(00)88205-2

Henrichs, J; Bongers-Schokking, JJ; Schenk, JJ; Ghassabian, A; Schmidt, HG; Visser, TJ; Hooijkaas, H; de Muinck Keizer-Schrama, SM; Hofman, A; Jaddoe, VV; Visser, W; Steegers, EA; Verhulst, FC; de Rijke, YB; Tiemeier, H. (2010). Maternal thyroid function during early pregnancy and cognitive functioning in early childhood: The generation R study. J Clin Endocrinol Metab 95: 4227-4234. http://dx.doi.org/10.1210/jc.2010-0415

Hinkson, NC; Whalen, MM. (2009). Hexabromocyclododecane decreases the lytic function and ATP levels of human natural killer cells. J Appl Toxicol 29: 656-661. http://dx.doi.org/10.1002/jat.1453

Hinkson, NC; Whalen, MM. (2010). Hexabromocyclododecane decreases tumor-cell-binding capacity and cell-surface protein expression of human natural killer cells. J Appl Toxicol 30: 302-309. http://dx.doi.org/10.1002/jat.1495

Howdeshell, KL. (2002). A model of the development of the brain as a construct of the thyroid system [Review]. Environ Health Perspect 110 Suppl 3: 337-348. http://dx.doi.org/10.1289/ehp.02110s3337

Hu, J; Liang, Y; Chen, M; Wang, X. (2009a). Assessing the toxicity of TBBPA and HBCD by zebrafish embryo toxicity assay and biomarker analysis. Environ Toxicol 24: 334-342. http://dx.doi.org/10.1002/tox.20436

Hu, X; Hu, D; Xu, Y. (2009b). Effects of tetrabrominated diphenyl ether and hexabromocyclododecanes in single and complex exposure to hepatoma HepG2 cells. Environ Toxicol Pharmacol 27: 327-337. http://dx.doi.org/10.1016/j.etap.2008.11.014

Huang, X; Chen, C; Shang, Y; Zhong, Y; Ren, G; Yu, Z; An, J. (2016). In vitro study on the biotransformation and cytotoxicity of three hexabromocyclododecane diastereoisomers in liver cells. Chemosphere 161: 251-258. http://dx.doi.org/10.1016/j.chemosphere.2016.07.001.

Huntingdon Research, C. (1990). Ames metabolic activation test to assess the potential mutagenic effect of und no. 49 with cover letter dated 031290 [TSCA Submission].

(TSCATS/406642. OTS0522948. 86900000385). Wyandotte, MI: BASF Corporation. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0522948
Ibhazehiebo, K; Iwasaki, T; Shimokawa, N; Koibuchi, N. (2011a). 1,2,5,6,9,10-αHexabromocyclododecane (HBCD) impairs thyroid hormone-induced dendrite arborization of Purkinje cells and suppresses thyroid hormone receptor-mediated transcription. Cerebellum 10: 22-31. http://dx.doi.org/10.1007/s12311-010-0218-1
Ibhazehiebo, K; Iwasaki, T; Xu, M; Shimokawa, N; Koibuchi, N. (2011b). Brain-derived neurotrophic factor (BDNF) ameliorates the suppression of thyroid hormone-induced granule cell neurite extension by hexabromocyclododecane (HBCD). Neurosci Lett 493: 1-7. http://dx.doi.org/10.1016/j.neulet.2011.01.062
International, SRI. (1990). In vitro microbiological mutagenicity studies of four Ciba-Geigy Corporation compounds (final report) with test data and cover letter [TSCA Submission]. (TSCATS/407254. OTS0523254. Doc I.D. 86900000262). West Lafayette, IN: Great Lakes Chemical Corporation. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0523254
Johnson, PI; Stapleton, HM; Mukherjee, B; Hauser, R; Meeker, JD. (2013). Associations between brominated flame retardants in house dust and hormone levels in men. Sci Total Environ 445-446: 177-184. http://dx.doi.org/10.1016/j.scitotenv.2012.12.017
Julvez, J; Alvarez-Pedrerol, Ma; Rebagliato, M; Murcia, M; Forns, J; Garcia-Esteban, R; Lertxundi, N; Espada, M; Tardon, A; Riano Galan, I; Sunyer, J. (2013). Thyroxine levels during pregnancy in healthy women and early child neurodevelopment. Epidemiology 24: 150-157. http://dx.doi.org/10.1097/EDE.0b013e318276ccd3
Kang, NH; Hwang, KA; Kim, TH; Hyun, SH; Jeung, EB; Choi, KC. (2012). Induced growth of BG-1 ovarian cancer cells by 17β-estradiol or various endocrine disrupting chemicals was reversed by resveratrol via downregulation of cell cycle progression. Mol Med Rep 6: 151-156. http://dx.doi.org/10.3892/mmr.2012.887
Kato, Y; Ikushiro, S; Emi, Y; Tamaki, S; Suzuki, H; Sakaki, T; Yamada, S; Degawa, M. (2008). Hepatic UDP-glucuronosyltransferases responsible for glucuronidation of thyroxine in humans. Drug Metab Dispos 36: 51-55. http://dx.doi.org/10.1124/dmd.107.018184
Kelly, GS. (2000). Peripheral metabolism of thyroid hormones: a review [Review]. Altern Med Rev 5: 306-333.
Kiciński, M; Viaene, MK; Den Hond, E; Schoeters, G; Covaci, A; Dirtu, AC; Nelen, V; Bruckers, L; Croes, K; Sioen, I; Baeyens, W; Van Larebeke, N; Nawrot, TS. (2012). Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: A cross-sectional study. Environ Health 11: 86. http://dx.doi.org/10.1186/1476-069X-11-86
Kim, SH; Nam, KH; Hwang, KA; Choi, KC. (2016). Influence of hexabromocyclododecane and 4-nonylphenol on the regulation of cell growth, apoptosis and migration in prostatic cancer cells. Toxicol In Vitro 32: 240-247. http://dx.doi.org/10.1016/j.tiv.2016.01.008
Kim, UJ; Oh, JE. (2014). Tetrabromobisphenol A and hexabromocyclododecane flame retardants in infant-mother paired serum samples, and their relationships with thyroid hormones and environmental factors. Environ Pollut 184: 193-200. http://dx.doi.org/10.1016/j.envpol.2013.08.034
Kitamura, S; Suzuki, T; Sanoh, S; Kohta, R; Jinno, N; Sugihara, K; Yoshihara, S; Fujimoto, N; Watanabe, H; Ohta, S. (2005). Comparative study of the endocrine-disrupting activity of

bisphenol A and 19 related compounds. Toxicol Sci 84: 249-259. http://dx.doi.org/10.1093/toxsci/kfi074
Klaassen, CD; Hood, AM. (2001). Effects of microsomal enzyme inducers on thyroid follicular cell proliferation and thyroid hormone metabolism [Review]. Toxicol Pathol 29: 34-40. http://dx.doi.org/10.1080/019262301301418838
Klammer, H; Schlecht, C; Wuttke, W; Schmutzler, C; Gotthardt, I; Köhrle, J; Jarry, H. (2007). Effects of a 5-day treatment with the UV-filter octyl-methoxycinnamate (OMC) on the function of the hypothalamo-pituitary-thyroid function in rats. Toxicology 238: 192-199. http://dx.doi.org/10.1016/j.tox.2007.06.088
Koibuchi, N; Chin, MW. (2000). Thyroid hormone action and brain development. Trends Endocrinol Metab 11: 123-128. http://dx.doi.org/10.1016/S1043-2760(00)00238-1
Koike, E; Yanagisawa, R; Takigami, H; Takano, H. (2012). Brominated flame retardants stimulate mouse immune cells in vitro. J Appl Toxicol 33: 1451-1459. http://dx.doi.org/10.1002/jat.2809
Kretschmer, XC; Baldwin, WS. (2005). CAR and PXR: xenosensors of endocrine disrupters? [Review]. Chem Biol Interact 155: 111-128. http://dx.doi.org/10.1016/j.cbi.2005.06.003
Krivoshiev, BV; Dardenne, F; Covaci, A; Blust, R; Husson, SJ. (2016). Assessing in-vitro estrogenic effects of currently-used flame retardants. Toxicol In Vitro 33: 153-162. http://dx.doi.org/10.1016/j.tiv.2016.03.006
Labs, IBT. (1990). Mutagenicity of two lots of FM-100 lot 53 and residue of lot 3322 in the absence and presence of metabolic activation with test data and cover letter [TSCA Submission] (pp. #86-900000267). (TSCATS/407259. OTS0523259. Doc I.D. 86900000267). West Lafayette, IN: Great Lakes Chemical Corporation. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0523259
Lanham, SA; Fowden, AL; Roberts, C; Cooper, C; Oreffo, ROC; Forhead, AJ. (2011). Effects of hypothyroidism on the structure and mechanical properties of bone in the ovine fetus. J Endocrinol 210: 189-198. http://dx.doi.org/10.1530/JOE-11-0138
Lans, MC; Klasson-Wehler, E; Willemsen, M; Meussen, E; Safe, S; Brouwer, A. (1993). Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-p-dioxins and -dibenzofurans with human transthyretin. Chem Biol Interact 88: 7-21. http://dx.doi.org/10.1016/0009-2797(93)90081-9
Laven, JS; Mulders, AG; Visser, JA; Themmen, AP; De Jong, FH; Fauser, BC. (2004). Anti-Müllerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. J Clin Endocrinol Metab 89: 318-323. http://dx.doi.org/10.1210/jc.2003-030932
Lilienthal, H; van der Ven, LT; Piersma, AH; Vos, JG. (2009). Effects of the brominated flame retardant hexabromocyclododecane (HBCD) on dopamine-dependent behavior and brainstem auditory evoked potentials in a one-generation reproduction study in Wistar rats. Toxicol Lett 185: 63-72. http://dx.doi.org/10.1016/j.toxlet.2008.12.002
Litton, B. (1990). Mutagenicity evaluation of 421-32B (final report) with test data and cover letter [TSCA Submission]. (TSCATS/407257. OTS0523257. 86900000265). West Lafayette, IN: Great Lakes Chemical Corporation. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0523257
Liu, D; Teng, W; Shan, Z; Yu, X; Gao, Y; Wang, S; Fan, C; Wang, H; Zhang, H. (2010). The effect of maternal subclinical hypothyroidism during pregnancy on brain development in rat offspring. Thyroid 20: 909-915. http://dx.doi.org/10.1089/thy.2009.0036

Luster, MI; Johnson, VJ; Yucesoy, B; Simeonova, PP. (2005). Biomarkers to assess potential developmental immunotoxicity in children. Toxicol Appl Pharmacol 206: 229-236. http://dx.doi.org/10.1016/j.taap.2005.02.010

Mackenzie, PI; Gregory, PA; Gardner-Stephen, DA; Lewinsky, RH; Jorgensen, BR; Nishiyama, T; Xie, W; Radominska-Pandya, A. (2003). Regulation of UDP glucuronosyltransferase genes [Review]. Curr Drug Metab 4: 249-257. http://dx.doi.org/10.2174/1389200033489442#sthash.z8bvGH58.dpuf

Makris, S; Makoto, E; Luke, A. (2016). Personal communication with Dr Makoto Ema [Personal Communication].

Maranghi, F; Tassinari, R; Moracci, G; Altieri, I; Rasinger, JD; Carroll, TS; Hogstrand, C; Lundebye, AK; Mantovani, A. (2013). Dietary exposure of juvenile female mice to polyhalogenated seafood contaminants (HBCD, BDE-47, PCB-153, TCDD): comparative assessment of effects in potential target tissues. Food Chem Toxicol 56: 443-449. http://dx.doi.org/10.1016/j.fct.2013.02.056

Mariussen, E; Fonnum, F. (2003). The effect of brominated flame retardants on neurotransmitter uptake into rat brain synaptosomes and vesicles. Neurochem Int 43: 533-542. http://dx.doi.org/10.1016/S0197-0186(03)00044-5

Maron, DM; Ames, BN. (1983). Revised methods for salmonella mutagenicity test. Mutat Res Environ Mutagen Relat Subj 113: 173-215. http://dx.doi.org/10.1016/0165-1161(83)90010-9

Meeker, JD; Singh, NP; Hauser, R. (2008). Serum concentrations of estradiol and free T4 are inversely correlated with sperm DNA damage in men from an infertility clinic. J Androl 29: 379-388. http://dx.doi.org/10.2164/jandrol.107.004416

Meijer, L; Martijn, A; Melessen, J; Brouwer, A; Weiss, J; de Jong, FH; Sauer, PJ. (2012). Influence of prenatal organohalogen levels on infant male sexual development: sex hormone levels, testes volume and penile length. Hum Reprod 27: 867-872. http://dx.doi.org/10.1093/humrep/der426

Microbiological, A. (1996). Hexabromocyclododecane (HBCD): Chromosome aberrations in human peripheral blood lymphocytes with cover letter dated 12/12/1996 [TSCA Submission]. (TSCATS/453439. OTS0573552. Doc I.D. 86970000358). Arlington, VA: Chemical Manufacturers Association. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0573552

Miller-Rhodes, P; Popescu, M; Goeke, C; Tirabassi, T; Johnson, L; Markowski, VP. (2014). Prenatal exposure to the brominated flame retardant hexabromocyclododecane (HBCD) impairs measures of sustained attention and increases age-related morbidity in the Long-Evans rat. Neurotoxicol Teratol 45: 34-43. http://dx.doi.org/10.1016/j.ntt.2014.06.009

Miller, MD; Crofton, KM; Rice, DC; Zoeller, RT. (2009). Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes [Review]. Environ Health Perspect 117: 1033-1041. http://dx.doi.org/10.1289/ehp.0800247

Momma, J; Kaniwa, M; Sekiguchi, H; Ohno, K; Kawasaki, Y; Tsuda, M; Nakamura, A; Kurokawa, Y. (1993). [Dermatological evaluation of a flame retardant, hexabromocyclododecane (HBCD) on guinea pig by using the primary irritation, sensitization, phototoxicity and photosensitization of skin]. Eisei Shikenjo Hokoku18-24.

Morse, DC; Groen, D; Veerman, M; van Amerongen, CJ; Koëter, HB; Smits van Prooije, AE; Visser, TJ; Koeman, JH; Brouwer, A. (1993). Interference of polychlorinated biphenyls

in hepatic and brain thyroid hormone metabolism in fetal and neonatal rats. Toxicol Appl Pharmacol 122: 27-33. http://dx.doi.org/10.1006/taap.1993.1168
Nakamura, A; Momma, J; Sekiguchi, H; Noda, T; Yamano, T; Kaniwa, M; Kojima, S; Tsuda, M; Kurokawa, Y. (1994). A new protocol and criteria for quantitative determination of sensitization potencies of chemicals by guinea pig maximization test. Contact Derm 31: 72-85. http://dx.doi.org/10.1111/j.1600-0536.1994.tb01921.x
Neher, E; Sakaba, T. (2008). Multiple roles of calcium ions in the regulation of neurotransmitter release. Neuron 59: 861-872. http://dx.doi.org/10.1016/j.neuron.2008.08.019
NRC. (2005). Health implications of perchlorate ingestion. Washington, DC: National Academies Press. http://dx.doi.org/https://doi.org/10.17226/11202
Ogaswara, S; Hanafusa, T. (1993). Report on mutagenicity test on Pyroguard SR-103 using microorganisms [unpublished]. Ogaswara, S; Hanafusa, T.
Omiecinski, CJ; Vanden Heuvel, JP; Perdew, GH; Peters, JM. (2011). Xenobiotic metabolism, disposition, and regulation by receptors: from biochemical phenomenon to predictors of major toxicities [Review]. Toxicol Sci 120: S49-S75. http://dx.doi.org/10.1093/toxsci/kfq338
Palace, V; Park, B; Pleskach, K; Gemmill, B; Tomy, G. (2010). Altered thyroxine metabolism in rainbow trout (Oncorhynchus mykiss) exposed to hexabromocyclododecane (HBCD). Chemosphere 80: 165-169. http://dx.doi.org/10.1016/j.chemosphere.2010.03.016
Palace, VP; Pleskach, K; Halldorson, T; Danell, R; Wautier, K; Evans, B; Alaee, M; Marvin, C; Tomy, GT. (2008). Biotransformation enzymes and thyroid axis disruption in juvenile rainbow trout (Oncorhynchus mykiss) exposed to hexabromocyclododecane diastereoisomers. Environ Sci Technol 42: 1967-1972. http://dx.doi.org/10.1021/es702565h
Park, MA; Hwang, KA; Lee, HR; Yi, BR; Jeung, EB; Choi, KC. (2012). Cell growth of BG-1 ovarian cancer cells is promoted by di-n-butyl phthalate and hexabromocyclododecane via upregulation of the cyclin D and cyclin-dependent kinase-4 genes. Mol Med Rep 5: 761-766. http://dx.doi.org/10.3892/mmr.2011.712
Patel, J; Landers, K; Li, H; Mortimer, RH; Richard, K. (2011). Thyroid hormones and fetal neurological development. J Endocrinol 209: 1-8. http://dx.doi.org/10.1530/JOE-10-0444
Pererira, DN; Procianoy, RS. (2003). Effect of perinatal asphyxia on thyroid-stimulating hormone and thyroid hormone levels. Acta Paediatr 92: 339-345. http://dx.doi.org/10.1111/j.1651-2227.2003.tb00556.x
Pharmakologisches, I. (1990). Ames test with hexabromides with cover letter dated 031290 [TSCA Submission]. (TSCATS/406636. OTS0522942. Doc. I.D. 86900000379). Washington, DC: U.S. Environmental Protection Agency.
Pucci, E; Chiovato, L; Pinchera, A. (2000). Thyroid and lipid metabolism. Int J Obes (Lond) 24: S109-S112.
Reistad, T; Fonnum, F; Mariussen, E. (2006). Neurotoxicity of the pentabrominated diphenyl ether mixture, DE-71, and hexabromocyclododecane (HBCD) in rat cerebellar granule cells in vitro. Arch Toxicol 80: 785-796. http://dx.doi.org/10.1007/s00204-006-0099-8
Rodriguez, MJ; Adroer, R; de Yebra, Ll; Ramonet, D; Mahy, N. (2001). Calcium homeostasis in the central nervous system: Adaptation to neurodegeneration. Contributions to Science 2: 43-61.

Román, GC; Ghassabian, A; Bongers-Schokking, JJ; Jaddoe, VW; Hofman, A; de Rijke, YB; Verhulst, FC; Tiemeier, H. (2013). Association of gestational maternal hypothyroxinemia and increased autism risk. Ann Neurol 74: 733-742. http://dx.doi.org/10.1002/ana.23976

Rosenfeld, JM; Vargas, R; Xie, W; Evans, RM. (2003). Genetic profiling defines the xenobiotic gene network controlled by the nuclear receptor pregnane X receptor. Mol Endocrinol 17: 1268-1282. http://dx.doi.org/10.1210/me.2002-0421

Rosol, TJ; DeLellis, RA; Harvey, PW; Sutcliffe, C. (2013). Endocrine system. In W Haschek; C Rousseaux; M Wallig (Eds.), (3rd ed., pp. 2391–2492). Waltham, MA: Academic Press. http://dx.doi.org/10.1016/B978-0-12-415759-0.00058-3

Roze, E; Meijer, L; Bakker, A; Van Braeckel, KNJA; Sauer, PJJ; Bos, AF. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age. Environ Health Perspect 117: 1953-1958. http://dx.doi.org/10.1289/ehp.0901015

Saegusa, Y; Fujimoto, H; Woo, GH; Inoue, K; Takahashi, M; Mitsumori, K; Hirose, M; Nishikawa, A; Shibutani, M. (2009). Developmental toxicity of brominated flame retardants, tetrabromobisphenol A and 1,2,5,6,9,10-hexabromocyclododecane, in rat offspring after maternal exposure from mid-gestation through lactation. Reprod Toxicol 28: 456-467. http://dx.doi.org/10.1016/j.reprotox.2009.06.011

Saegusa, Y; Fujimoto, H; Woo, GH; Ohishi, T; Wang, L; Mitsumori, K; Nishikawa, A; Shibutani, M. (2012). Transient aberration of neuronal development in the hippocampal dentate gyrus after developmental exposure to brominated flame retardants in rats. Arch Toxicol 86: 1431-1442. http://dx.doi.org/10.1007/s00204-012-0824-4

Sakai, H; Kim, EY; Petrov, EA; Tanabe, S; Iwata, H. (2009). Transactivation potencies of Baikal seal constitutive active/androstane receptor by persistent organic pollutants and brominated flame retardants. Environ Sci Technol 43: 6391-6397. http://dx.doi.org/10.1021/es901120r

Schriks, M; Roessig, JM; Murk, AJ; Furlow, JD. (2007). Thyroid hormone receptor isoform selectivity of thyroid hormone disrupting compounds quantified with an in vitro reporter gene assay. Environ Toxicol Pharmacol 23: 302-307. http://dx.doi.org/10.1016/j.etap.2006.11.007

Schriks, M; Vrabie, CM; Gutleb, AC; Faassen, EJ; Rietjens, IM; Murk, AJ. (2006a). T-screen to quantify functional potentiating, antagonistic and thyroid hormone-like activities of poly halogenated aromatic hydrocarbons (PHAHs). Toxicol In Vitro 20: 490-498. http://dx.doi.org/10.1016/j.tiv.2005.09.001

Schriks, M; Zvinavashe, E; Furlow, JD; Murk, AJ. (2006b). Disruption of thyroid hormone-mediated Xenopus laevis tadpole tail tip regression by hexabromocyclododecane (HBCD) and 2,2',3,3',4,4',5,5',6-nona brominated diphenyl ether (BDE206). Chemosphere 65: 1904-1908. http://dx.doi.org/10.1016/j.chemosphere.2006.07.077

Schussler, GC. (2000). The thyroxine-binding proteins [Review]. Thyroid 10: 141-149. http://dx.doi.org/10.1089/thy.2000.10.141

Scott, HM; Mason, JI; Sharpe, RM. (2009). Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds [Review]. Endocr Rev 30: 883-925. http://dx.doi.org/10.1210/er.2009-0016

Shaffer, BM. (1963). The isolated Xenopus laevis tail: a preparation for studying the central nervous system and metamorphosis in culture. J Embryol Exp Morphol 11: 77-90.

Shelby, MK; Cherrington, NJ; Vansell, NR; Klaassen, CD. (2003). Tissue mRNA expression of the rat UDP-glucuronosyltransferase gene family. Drug Metab Dispos 31: 326-333. http://dx.doi.org/10.1124/dmd.31.3.326

Shields, BM; Knight, BA; Hill, A; Hattersley, AT; Vaidya, B. (2011). Fetal thyroid hormone level at birth is associated with fetal growth. J Clin Endocrinol Metab 96: E934-E938. http://dx.doi.org/10.1210/jc.2010-2814

Steinmaus, C; Miller, MD; Cushing, L; Blount, BC; Smith, AH. (2013). Combined effects of perchlorate, thiocyanate, and iodine on thyroid function in the National Health and Nutrition Examination Survey 2007-08. Environ Res 123: 17-24. http://dx.doi.org/10.1016/j.envres.2013.01.005

Tonacchera, M; Pinchera, A; Dimida, A; Ferrarini, E; Agretti, P; Vitti, P; Santini, F; Crump, K; Gibbs, J. (2004). Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter. Thyroid 14: 1012-1019. http://dx.doi.org/10.1089/thy.2004.14.1012

U.S, EPA. (1996). Guidelines for reproductive toxicity risk assessment (pp. 1-143). (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2014-11/documents/guidelines_repro_toxicity.pdf

U.S, EPA. (2012). Benchmark dose technical guidance. (EPA/100/R-12/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/risk/benchmark-dose-technical-guidance

Ueda, A; Hamadeh, HK; Webb, HK; Yamamoto, Y; Sueyoshi, T; Afshari, CA; Lehmann, JM; Negishi, M. (2002). Diverse roles of the nuclear orphan receptor CAR in regulating hepatic genes in response to phenobarbital. Mol Pharmacol 61: 1-6. http://dx.doi.org/10.1124/mol.61.1.1

van den Dungen, MW; Rijk, JC; Kampman, E; Steegenga, WT; Murk, AJ. (2015). Steroid hormone related effects of marine persistent organic pollutants in human H295R adrenocortical carcinoma cells. Toxicol In Vitro 29: 769-778. http://dx.doi.org/10.1016/j.tiv.2015.03.002

van der Ven, LT; Verhoef, A; van de Kuil, T; Slob, W; Leonards, PE; Visser, TJ; Hamers, T; Herlin, M; Håkansson, H; Olausson, H; Piersma, AH; Vos, JG. (2006). A 28-day oral dose toxicity study enhanced to detect endocrine effects of hexabromocyclododecane in Wistar rats. Toxicol Sci 94: 281-292. http://dx.doi.org/10.1093/toxsci/kfl113

van der Ven, LTM; van de Kuil, T; Leonards, PEG; Slob, W; Lilienthal, H; Litens, S; Herlin, M; Håkansson, H; Cantón, RF; van den Berg, M; Visser, TJ; van Loveren, H; Vos, JG; Piersma, AH. (2009). Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats. Toxicol Lett 185: 51-62. http://dx.doi.org/10.1016/j.toxlet.2008.12.003

Vansell, NR; Klaassen, CD. (2002). Effect of microsomal enzyme inducers on the biliary excretion of triiodothyronine (T(3)) and its metabolites. Toxicol Sci 65: 184-191. http://dx.doi.org/10.1093/toxsci/65.2.184

Waner, T; Nyska, A. (1991). The toxicological significance of decreased activities of blood alanine and aspartate aminotransferase [Review]. Vet Res Commun 15: 73-78. http://dx.doi.org/10.1007/BF00497793

Watanabe, W; Shimizu, T; Sawamura, R; Hino, A; Konno, K; Hirose, A; Kurokawa, M. (2010). Effects of tetrabromobisphenol A, a brominated flame retardant, on the immune response

to respiratory syncytial virus infection in mice. Int Immunopharmacol 10: 393-397. http://dx.doi.org/10.1016/j.intimp.2009.12.014
Wheater, PR; Burkitt, HG. (1996). Wheater's basic histopathology: a colour atlas and text. New York: Churchill Livingstone.
Who. (2012). Guidance for immunotoxicity risk assessment for chemicals. In IPCS Harmonization Project. (Harmonization Project Document No. 10). Geneva, Switzerland. http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf
WIL Research. (1997). Twenty-eight day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997 [TSCA Submission]. (TSCATS/445005. OTS0558957. Doc I.D. #86970000747). Washington, DC: Chemical Manufacturers Association. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0558957
WIL Research. (2001). 90-Day oral (gavage) toxicity study of HBCD in rats. (WIL-186012). Washington, DC: Chemical Manufacturers Association.
Wu, M; Wu, D; Wang, C; Guo, Z; Li, B; Zuo, Z. (2016). Hexabromocyclododecane exposure induces cardiac hypertrophy and arrhythmia by inhibiting miR-1 expression via up-regulation of the homeobox gene Nkx2.5. J Hazard Mater 302: 304-313. http://dx.doi.org/10.1016/j.jhazmat.2015.10.004
Wu, M; Zuo, Z; Li, B; Huang, L; Chen, M; Wang, C. (2013). Effects of low-level hexabromocyclododecane (HBCD) exposure on cardiac development in zebrafish embryos. Ecotoxicology 22: 1200-1207. http://dx.doi.org/10.1007/s10646-013-1107-4
Yamada-Okabe, T; Sakai, H; Kashima, Y; Yamada-Okabe, H. (2005). Modulation at a cellular level of the thyroid hormone receptor-mediated gene expression by 1,2,5,6,9,10-hexabromocyclododecane (HBCD), 4,4'-diiodobiphenyl (DIB), and nitrofen (NIP). Toxicol Lett 155: 127-133. http://dx.doi.org/10.1016/j.toxlet.2004.09.005
Yanagisawa, R; Koike, E; Win-Shwe, TT; Yamamoto, M; Takano, H. (2014). Impaired lipid and glucose homeostasis in hexabromocyclododecane-exposed mice fed a high-fat diet. Environ Health Perspect 122: 277-283. http://dx.doi.org/10.1289/ehp.1307421
Zeiger, E; Anderson, B; Haworth, S; Lawlor, T; Mortelmans, K; Speck, W. (1987). Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals. Environ Mutagen 9: 1-109. http://dx.doi.org/10.1002/em.2860090602
Zhang, X; Yang, F; Zhang, X; Xu, Y; Liao, T; Song, S; Wang, J. (2008). Induction of hepatic enzymes and oxidative stress in Chinese rare minnow (Gobiocypris rarus) exposed to waterborne hexabromocyclododecane (HBCDD). Aquat Toxicol 86: 4-11. http://dx.doi.org/10.1016/j.aquatox.2007.07.002

Zoeller, RT; Tan, SW; Tyl, RW. (2007). General background on the hypothalamic-pituitary-thyroid (HPT) axis [Review]. Crit Rev Toxicol 37: 11-53. http://dx.doi.org/10.1080/10408440601123446