UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
Bethesda, MD 20814

Memorandum

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SUBJECT : Toxicity Review of **Diisobutyl phthalate (DiBP)**

The following memo provides the Versar Inc. and SRC, Inc. contractor's and U.S. Consumer Product Safety Commission's (CPSC's) Health Sciences staff assessment of the potential toxicity associated with **DiBP**.

CPSC staff assesses a product's potential health effects to consumers under the Federal Hazardous Substances Act (FHSA). The FHSA is risk-based. To be considered a "hazardous substance" under the FHSA, a consumer product must satisfy a two-part definition. First, it must be "toxic" under the FHSA, or present one of the other hazards enumerated in the statute. Second, it must have the potential to cause "substantial illness or injury during or as a result of reasonably foreseeable handling or use." Therefore, exposure and risk must be considered in addition to toxicity when assessing potential hazards under the FHSA (CPSC, 1992; summarized at 16 CFR 1500.135)

The FHSA addresses both acute and chronic hazards. While the FHSA does not require manufacturers to perform any specific battery of toxicological tests to assess the potential risk of chronic health hazards, the manufacturer is required to label a product appropriately according to the requirements of the FHSA. The first step in the risk assessment process is hazard

* This report was prepared for the Commission pursuant to contract CPSC-D-06-0006. It has not been reviewed or approved by, and may not necessarily reflect the views of, the Commission

identification, that is, a review of the available toxicity data for the chemical under consideration and a determination of whether the chemical is considered “toxic”. Chronic toxicity data (including carcinogenicity, neurotoxicity, and reproductive and developmental toxicity) are assessed by the CPSC staff using guidelines issued by the Commission (CPSC, 1992). If it is concluded that a substance is “toxic” due to chronic toxicity, then a quantitative assessment of exposure and risk is performed to evaluate whether the chemical may be considered a “hazardous substance”. This memo represents the first step in the risk assessment process; that is, the hazard identification step.

FINAL

TOXICITY REVIEW FOR DIISOBUTYL PHTHALATE (DiBP, CASRN 84-69-5)

Contract No. CPSC-D-06-0006
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LIST OF ABBREVIATIONS AND ACRONYMS

AGD	Anogenital distance
AGI	Anogenital index
DiBP	Diisobutyl phthalate
GD	Gestation day
Hgb	Hemoglobin
i.p.	Intraperitoneal
LOAEL	Lowest-observed-adverse-effect level
LD_{50}	Median lethal dose
MIBP	Monoisobutyl phthalate
NOAEL	No-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
PND	Postnatal day
PNW	Postnatal week
PPS	Preputial separation
RBC	Red blood cell
SD	Standard deviation
SE	Standard error
TTM	Transabdominal testicular migration
TUNEL	Transferase dUTP nick end labeling
WBC	White blood cell

EXECUTIVE SUMMARY

DiBP is a minor use plasticizer found in a variety of consumer products.

Exposure to DiBP resulted in oral LD_{50}s >10,000 mg/kg in multiple animal studies. Insufficient data were available to make the determination of whether DiBP was associated with acute dermal or inhalation toxicity, eye or dermal irritation, or sensitization.

Evidence supported the conclusion that DiBP was a subchronic toxicant. Exposure to DiBP induced changes in animal body weight, liver weight, and reproduction and development (testicular weight, spermatogenesis, fetal body weight, anogenital distance, testicular testosterone production, sertoli cell vacuolization, testicular development, and external malformations in reproductive tissue). There was inadequate evidence to support the conclusion that DiBP was a neurotoxicant.

Acceptable daily intake values (ADI's) are calculated when a given chemical is considered "toxic" and sufficient toxicity information is available. The ADI is the amount of a chemical that one may be exposed to on a daily basis without posing a significant risk of health effects to consumers.

ADI's were estimated for relevant exposure durations to DiBP for the general population and for other sensitive subpopulations because data on toxicological endpoints were corroborated in multiple quality studies.

In summary, data support the conclusion that DiBP can be considered "toxic" under the FHSA due to its toxicity following short-term and intermediate-term exposures. This conclusion was based on the sufficient evidence in animals of DiBP-induced toxicity to the liver, testes, fetus, and other tissues.

When considering FHSA criteria, products that contain DiBP may be considered "hazardous" if short-term exposures to the general population during "reasonably foreseeable handling and use" exceed the short-term ADI for the general population (0.14 mg DiBP/kg bw-day).

In addition, products that contain DiBP may be considered "hazardous" if short-term exposures during "reasonably foreseeable handling and use" exceed the short-term ADI for reproductive effects (0.85 DiBP/kg bw-day).

In addition, products that contain DiBP may be considered “hazardous” if exposures to reproductively viable female populations (13 to 49 years of age) during “reasonably foreseeable handling and use” exceed the ADI for developmental effects (0.098 mg DiBP/kg bw-day).

Insufficient evidence (hazard data) precluded the generation of ADI’s for inhalation or dermal exposures or for cancer endpoints.

TOXICITY REVIEW FOR DIISOBUTYL PHTHALATE (DiBP, CASRN 84-69-5)

1. INTRODUCTION

This report summarizes available data on the identity, physicochemical properties, manufacture, supply, use, toxicity, and exposure associated with diisobutyl phthalate (DiBP). This assessment was prepared from a variety of review articles (NICNAS, 2008; ECB, 2000; ECHA, 2009) as well as supplemental independent studies retrieved from literature searching.

Historically, concerns regarding most phthalates have been primarily associated with their potential to induce adverse reproductive/developmental effects in humans (NICNAS, 2008). The structural and physicochemical properties of certain phthalates that allow migration and leaching out of products, especially soft plastics, have also been a concern (NICNAS, 2008).

2. PHYSICOCHEMICAL CHARACTERISTICS

This section highlights the identity and key physicochemical properties of DiBP.

The physicochemical and toxicological properties of phthalates are affected by structural characteristics of the side chains. Reproductive and developmental toxicity appear to be predominantly associated with phthalates having a carbon backbone of C3 up to C6 (ECHA, 2009). DiBP is considered a low molecular weight phthalate with a carbon backbone of C3. The linear portion of the carbon side chain is three carbon atoms in length (branched at C2).

The identity and physicochemical properties of DiBP can be seen in Tables 2.1 and 2.2 (NICNAS, 2008; HSDB, 2009; ECB, 2000).

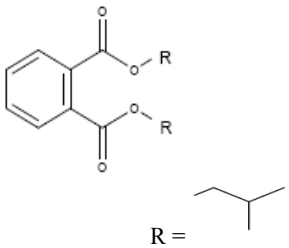
Table 2.1 Names, Structural Descriptors, and Molecular Formulas of DiBP (NICNAS, 2008)	
CAS Number:	84-69-5
Chemical Name:	1,2-Benzenedicarboxylic acid, bis-(2-methoxypropyl) ester
Common Name:	Diisobutyl phthalate (DiBP).
Molecular Formula:	C16H22O4
Structural Formula:	 R =
Molecular Weight:	278.35
Synonyms:	Phthalic acid, diisobutyl ester; Di(isobutyl)-,2 benzenedicarboxylate 1,2-Benzenedicarboxylic acid, bis-(2-methylpropyl) ester (ECB, 2000)
Purity/Impurities/Additives:	Purity >99%

Table 2.2 Physicochemical Properties of DiBP	
Property	**Value**
Physical state	Colorless, clear, mostly odorless, viscous liquid (NICNAS, 2008; ECHA, 2009)
Melting point	-37°C (NICNAS, 2008); -64°C (HSDB, 2009; ECB, 2000); -50°C, -42°C (ECB, 2000)
Boiling point	320 °C (NICNAS, 2008; ECHA 2009); 296.5 °C (HSDB, 2009); 295 -327°C (ECB, 2000)
Density	1038 kg/m^3 (NICNAS, 2008); 1.0409 (15°C) (HSDB, 2009); 1.037 - 1.049 (20°C) (ECB, 2000)
Vapor pressure	1.0 x 10^{-5} kPa (20°C) (NICNAS, 2008); 4.76 x 10^{-5} mm Hg (25°C) (HSDB, 2009)
Water solubility	1.1 x 10^{-3} g/L (NICNAS, 2008); 6.2 g/L (24°C) (HSDB, 2009); 20 mg/L (20°C) (ECHA, 2009)
Partition coefficient n-octanol/water (log Kow)	4.11 (NICNAS, 2008; HSDB, 2009; ECB 2000; ECHA, 2009)
Henry's law constant	6.43 x 10^{-7} atm-m^3/mole (NICNAS, 2008); 1.22 x 10^{-6} (25°C) (HSDB, 2009)
Flash point (closed cup)	185°C (NICNAS, 2008; HSDB, 2009); 161 - 185°C (ECB,2000)

3. MANUFACTURE, SUPPLY, AND USE

Manufacture

In general, DiBP is manufactured commercially in a closed system by catalytically esterifying phthalic anhydride with n-butyl alcohols (isobutanol). As with other phthalates, the unreacted alcohols are recovered and reused, and the DiBP mixture is purified by vacuum distillation or activated charcoal. The purity of DiBP can achieve 99% or greater using current manufacturing processes. The remaining fraction of DiBP may contain a maximum of 0.1% water.

DiBP is currently manufactured in Tennessee (Eastman Chemical Company) and North Carolina (Unitex Chemical Company under the trade name Uniplex 155). Eastman Chemical Company will be discontinuing dibutyl phthalate (DBP) (and presumably DiBP) production, however, in December of 2011.

Supply

U.S. production of DiBP is low and has been combined with several other phthalates (benzyl, undecyl dodecyl, n-butyl cyclohexyl, cyclohexyl, n-butyl-2-ethylhexyl, dicapryl, isooctyl isodecyl, diethylene glycol, and cyclohexyl-2-ethylhexyl phthalate) in marketing reports (Bizzari et al. 2009). Historically, combination production of these phthalates has increased from 5,000 (1982) to 13,000 metric tons (2004).

U.S. consumption of DiBP is low and has been combined with several other phthalates (undecyl dodecyl, n-butyl cyclohexyl, cyclohexyl, n-butyl-2-ethylhexyl, isooctyl isodecyl, diethylene glycol, isooctyl diphenyl, cyclohexyl-2-ethylhexyl, and di-(butoxyethyl) phthalate) in marketing reports (Bizzari et al. 2009). Historically, the combined production of these phthalates has increased from 5,000 (1982) to 14,000 metric tons (2004).

Marketing data suggest that U.S. consumption (in metric tons) of DiBP has been slightly higher than production, meaning that DiBP produced in the U.S. is probably utilized locally and also that a small amount of DiBP may be imported.

The U.S. production range in 2002 was > 500,000 - 1 million pounds based on the non-confidential production volume information submitted under Inventory Update Rule (IUR) (HSDB, 2009).

The world-wide production of both DBP and DiBP was estimated at 450,000 tons/year (ECHA, 2009). In an authorized IUCLID data sheet the quantity of DiBP manufactured and/or used in Europe is indicated in the range of 10,000 to 50,000 tons/year (ECHA, 2009)

Use

DiBP is considered a specialty plasticizer that is too volatile for use in PVC; it is often combined with other phthalates (U.S. EPA, 2009). It has good heat and light stability and has been used as a plasticizer for nitrocellulose (lowest cost plasticizer for cellulose nitrate), cellulose ether, and polyacrylate and polyacetate dispersions (U.S. EPA, 2009; HSDB 2009; ECHA, 2009). It is used in nail polish, cosmetics, lubricants, floor carpets, tapestry, clothing treatments, rubber dentistry settings, as a fuel stabilizer, in leather varnishes and lacquers, as a concrete additive, as an adjusting agent for lead chromate paint pigments, explosive material, lacquer manufacturing, and methyl methacrylate applications. DiBP is also used in printing inks for paper and packaging (ECHA, 2009). Because DiBP has similar properties as dibutyl phthalate (DBP), it can be used as a substitute for DBP (ECHA, 2009).

In Australia, DiBP is imported for use a plasticizer in the manufacture of PVC and rubber and as a component of industrial adhesives and catalyst systems for polypropylene and fiberglass manufacture. It is also sold in Australia to various institutions and laboratories for research and product development (NICNAS, 2008).

4. TOXICOKINETICS

4.1. Absorption

Biomonitoring studies reporting detectable levels of the DiBP metabolite, monoisobutyl phthalate (MIBP), in human urine samples suggest absorption and metabolism of DiBP in humans (these studies are described in further detail in Section 4.3, Metabolism), but no studies were located that provide quantitative information on the rate or extent of absorption of DiBP in humans. Additionally, there is no information on the absorption of DiBP following oral or inhalation exposure in animals. In the one animal study available, Elisi et al. (1989) compared

the dermal absorption of several phthalate diesters in rats. In this study, single doses of 30–40 mg/kg of various [^{14}C]-labeled phthalate diesters, including DiBP, were applied in an ethanol solution to the clipped skin of male F344 rats (n=3); the authors reported that the dosing corresponded to approximately 5–8 mg/cm^2 skin (157 µmol/kg). The [^{14}C]-labeled compounds were uniformly labeled on the aromatic ring. After the ethanol evaporated, the treated skin was covered with a circular plastic cap that had been perforated for aeration. Every 24 hours for 7 days, urine and feces were collected for measurement of radioactivity. On the 7th day, the animals were sacrificed, and radioactivity in several organs including the skin was measured. The chemical nature of the radioactivity in the collected excreta and tissues was not characterized in this study.

Elsisi et al. (1989) reported that as the length of the alkyl side chain increased, the amount excreted in urine in the first 24 hours decreased. During the first 24-hour period, about 6.5 and 1% of the administered dose of DiBP were excreted in the urine and feces, respectively. The daily rate of excretion of DiBP in the urine and feces was fairly constant over the first four 24-hour collection periods and showed some marginal slowing in the last three collection periods. The 7-day cumulative dose excreted in urine and feces for DiBP was approximately 51%. Upon sacrifice 7 days after dermal application, the brain, spinal cord, lung, liver, spleen, intestine, kidney, testis, fat, muscle, and blood were removed for determination of radioactivity. The amounts of radioactivity in the selected organs, reported as percentages (± standard deviation [SD]) of the applied dose, were 0.11% ± 0.03 in adipose tissue, 0.22% ± 0.08 in muscle, and <0.5% for all other tissues combined. Radioactivity recovered from the treated skin accounted for 35% ± 13 of the applied dose, while 0.2% ± 0.1 was recovered from untreated skin and 6.0% ± 0.5 was recovered in the plastic cap used to cover the exposed area. Including the excreted DiBP (51% over 7 days), the total recovered amount was 93% ± 7. From these data, it is estimated that about 59% of the applied dose was absorbed by the skin over the 7-day period ([51.3% in urine, feces, and tissues] ÷ [93% recovered – 6% detected in plastic cap]). These results indicate that DiBP is extensively absorbed by the skin under occluded conditions.

4.2. Distribution

A Polish study, for which an English abstract is available, reported concentrations of DiBP in 15 human blood samples ranging from 10 to 63 ng/g blood (Strucinski et al., 2006), but further information on the distribution of DiBP in humans is not available. Elisi et al. (1989) reported 0.22 and 0.11% of the applied single dose of 157 µmol/kg [^{14}C]-DiBP in muscle and adipose tissue, respectively, in rats following dermal exposure (study details provided above in

Section 4.1, Absorption). Less than 0.5% of the applied dose was detected in all other examined tissues combined, suggesting limited accumulation of DiBP in non-portal-of- entry tissues in rats following dermal absorption.

4.3. Metabolism

The metabolism of DiBP has not been studied extensively. Mentlein and Butte (1989) demonstrated that diester phthalates are hydrolyzed by human and rat hepatic esterases to their corresponding monophthalate esters. In the case of DiBP, the liver rat esterase pI 6.2/6.4 was more active than the human esterase in hydrolyzing DiBP to MIBP (specific activity of 7.4 µmol/minute/mg protein using the rat esterase compared with 1.2 µmol/minute/mg protein with the human esterase). This study found that the Michaelis-Menten constant (K_m) decreased with increasing lipophilicity for the phthalates tested. Kinetic constants for DiBP by purified rat liver carboxylesterases ranged from 0.15 to 0.19 mM for K_m and from 0.76 to 7.68 µmol/ minute/mg for maximum velocity of enzyme reaction (V_{max}). The rat liver esterase of pI 6.2/6.4 hydrolyzed DiBP more quickly than the structurally similar dibutyl phthalate.

A number of studies (CDC, 2009; Hines et al., 2009; Seckin et al., 2009; Witassek et al., 2009, 2007; Ye et al., 2009, 2008; Adibi et al., 2008; Witassek and Angerer, 2008; Fromme et al., 2007a, b; Marsee et al., 2006; Swan et al., 2006, 2005) have assessed urinary levels of phthalate metabolites in human populations without clearly-defined sources of exposure to various phthalates. These studies detected MIBP, the only clearly identified metabolite of DiBP, in urine samples collected from people in various populations (general, industrial, pregnant women). Witassek et al. (2009) observed a correlation between the presence of MIBP in maternal urine and paired samples of amniotic fluid ($r=0.93$, $p < 0.001$) collected from pregnant female patients at a hospital in Germany. Additionally, some studies have reported detectable concentrations of MIBP in the breast milk of lactating mothers in Europe (Latini et al., 2009; Högberg et al., 2008), although Calafat et al. (2004) did not detect MIBP among pooled breast milk samples from women in the United States.

4.4. Elimination

As described above (under the "Metabolism" section), the metabolite MIBP has been detected in urine samples from humans in numerous studies, suggesting elimination of DiBP through urine. However, no studies measuring DiBP in human urine samples or DiBP or MIBP in exhaled breath or feces of humans are available.

Results from a study of dermally-exposed rats indicate that, following dermal absorption of ring-labeled [^{14}C]-DiBP, absorbed radioactivity is efficiently eliminated via the urine (the predominant route) and the feces, and that neither DiBP nor its metabolites accumulate in fat, muscle, brain, spinal cord, testis, or other tissues (Elsisi et al., 1989). Demonstrating the importance of urinary excretion versus fecal excretion, about 6.5 and 1% of the administered dose of DiBP in this study was excreted in the urine and feces, respectively, during the first 24-hour period of exposure.

4.5. Toxicokinetic Conclusion

No data were located on the kinetics of absorption, distribution, or elimination of DiBP in humans or animals following oral or inhalation exposure. A study of rats dermally exposed to DiBP indicated extensive absorption through the skin and rapid elimination in the urine and feces, without significant accumulation in non-portal-of-entry tissues. Although the metabolism of DiBP has not been studied extensively, an in vitro study with isolated rat and human esterases identified MIBP as a metabolite of DiBP. MIBP has been detected in various biological fluids (urine, amniotic fluid, breast milk) in human populations.

5. HAZARD INFORMATION

This section contains brief hazard summaries of the adverse effects of DiBP in a variety of animal and bacterial species. More detailed discussions of the studies can be viewed in the Appendices. When evaluating hazard study data, Consumer Product Safety Commission (CPSC) staff utilized the definitions for toxicity as presented in regulations (16 CFR §1500.3(c)(2)(ii)) and the chronic hazard guidelines (16 CFR §1500.135) in the Federal Hazardous Substances Act (FHSA; 15 U.S.C. 1261-1278). When considering the FHSA, substances that are "known" or "probable" toxicants are "toxic" and substances that are considered "possible" toxicants are "not toxic" (Table 5.1).

Table 5.1. Classification of Chronic Hazards (as per the FHSA)

Evidence	Human Studies	Animal Studies
Sufficient evidence	**Known**	**Probable**
Limited evidence	**Probable**	Possible
Inadequate evidence	Possible	—

Exposure to DiBP resulted in oral LD_{50}s >10,000 mg/kg in multiple animal studies. Insufficient data were available to make the determination of whether DiBP was associated with acute dermal or inhalation toxicity, eye or dermal irritation, or sensitization.

Evidence supported the conclusion that DiBP was a subchronic toxicant. Exposure to DiBP induced changes in body weight, liver weight, reproductive effects, and developmental effects (testicular weight, spermatogenesis, fetal body weight, anogenital distance in males and female rats, testicular testosterone production, sertoli cell vacuolization, impaired testicular development, and an increase in external malformations in reproductive tissue).

Acceptable daily intakes values (ADI's) are calculated when a given chemical is considered "toxic" and sufficient toxicity information is available. The ADI is the amount of a chemical that one may be exposed to on a daily basis without posing a significant risk of health effects to consumers.

ADI's were estimated for relevant exposure durations to DiBP for the general population and for other sensitive subpopulations because data on toxicological endpoints were corroborated in multiple quality studies.

The Benchmark Dose (BMD) methodology as discussed in Babich (2008) was used to determine an estimate of dose levels for particular adverse responses to DiBP (i.e. decreased body weight, increased relative liver weight, decreased ano-genital distance). Specifically, the 95% lower confidence level of the dose with a risk over background of 10% ($BMDL_{10}$) was calculated for all continuous and dichotomous data and endpoints. Select $BMDL_{10}$s were then used to calculate respective ADIs.

In the following discussions, hazard information was divided into sections thought to be of interest for regulatory matters (i.e., for labeling and other mitigation measures) as well as for biological and pathological consistency. More specifically, hazards were divided into whether the exposure was singular or repeated. Hazards associated with repeated exposures were further divided into groupings based on the affected organ system (i.e., hepatic, neurological, hematologic, etc.) and discussed in terms of the exposure duration if sufficient information existed to do so (***acute***, ≤14 days; ***intermediate-term*** or ***subchronic***, 15–364 days; ***long-term*** or ***chronic***, ≥365 days; and ***multigenerational***; ATSDR, 2007) where appropriate. Discrete study information can be reviewed in the Appendices.

ACUTE DOSE TOXICITY

5.1. Acute Oral Toxicity

Hodge (1954) investigated the acute toxicity of DiBP (purity not reported) following single oral (gavage or capsule) doses in rats (albino), mice (young adults), rabbits, guinea pigs, and cats (strains not reported). Multiple dose levels were tested in groups of 2–3 rats or mice, and LD_{50} values were determined based on 24-hour mortalities. Oral LD_{50} values were approximately 60,000 and 39,500 mg/kg in rats and mice, respectively. Dose groups of rabbits, guinea pigs, and cats comprised only a single animal each. The highest doses given orally that did not result in mortality during the 24 hours following dosing in these animals were approximately 17,500, 16,500, and 13,500 mg/kg in guinea pigs, rabbits, and cats, respectively. Late deaths among all species were usually observed at 48–72 hours after initial dosing, but occasional late deaths were observed on the 3rd or 4th day of the 14-day observation period. Histological examination of liver and kidney sections removed from guinea pigs, rabbits, and cats surviving the 2-week observation period revealed occasional minor pathological changes described by the study authors as comparable to historical controls. In general, there was little species variation in susceptibility, and the acute toxic dose of DiBP was about the same (on a body weight basis) for all of the species tested.

Eastman Kodak Co. (1978) provides a summary of acute toxicity testing results in rats and mice (number of animals tested not reported) following administration of single oral doses of DiBP (purity not reported) based on unpublished data. The toxicity summary describes DiBP as being slightly toxic to rats and mice and identifies oral LD_{50} values of 16,000–28,000 and >12,800 mg/kg, respectively. No further information is provided. Information on the acute toxicity of DiBP summarized by the European Commission (2004, 2000) based on unpublished data includes similar findings as described above, which suggest that DiBP is lethal at high doses to animals orally (LD_{50} values of 10,400–15,000 mg/kg in rats and 10,000–12,800 mg/kg in mice; number of animals tested not reported).

The estimated LD_{50}'s from the Hodge (1954), Eastman Kodak Co. (1978), and European Commission (2004, 2000) studies in rats, mice, rabbits, guinea pigs, and cats were all higher than the oral LD_{50} range (50–5,000 mg/kg) required by the FHSA to conclude that a chemical is acutely toxic (16 CFR §1500.3(c)(2)(i)(A)).

5.2. Acute Dermal Toxicity

The European Commission (2004, 2000) reported an acute dermal LD_{50} for DiBP (purity not reported) of 10,400 mg/kg in guinea pigs (number of animals tested not reported) based on unpublished data.

The lack of additional acute dermal toxicity data and methodological descriptions for DiBP is considered a data gap and supports the conclusion that there is "inadequate evidence" for the designation of DiBP as "acutely toxic" via dermal exposure under the FHSA (16 CFR §1500.3(c)(2)(i)(C)).

5.3. Acute Inhalation Toxicity

The European Commission (2004, 2000) reported no lethality among rats (n=6) exposed to DiBP (purity not reported) for 8 hours in an atmosphere saturated with the test substance at 20°C, but no other information was provided.

The lack of additional acute inhalation toxicity data and methodological descriptions for DiBP can be considered a data gap and supports the conclusion that there is "inadequate evidence" for the designation of DiBP as "acutely toxic" via inhalation under the FHSA (16 CFR §1500.3(c)(2)(i)(B)).

5.4. Acute Toxicity – Other Routes

Lawrence et al. (1975) calculated an intraperitoneal (i.p.) LD_{50} for DiBP in mice (n=10 per group, number of dose levels not specified) of 3,840 µL/kg or 3,990 mg/kg. The same group of researchers (Singh et al., 1972) reported an i.p. LD_{50} for DiBP in rats (group sizes and number of dose levels not specified) of 3,750 µL/kg (approximately 3,900 mg/kg). Eastman Kodak Co. (1978) provided a summary of acute toxicity testing results in rats and mice following administration of single i.p. doses of DiBP (purity not reported) based on unpublished data. The toxicity summary describes DiBP as being slightly toxic to rats and mice and identifies i.p. LD_{50} values of >1,600 and 6,400–12,800 mg/kg, respectively. No further information was provided. The European Commission (2004, 2000) reported i.p. LD_{50} values of ≥1,600 mg/kg in rats and mice.

In addition to evaluating the acute oral toxicity of DiBP as described above, Hodge (1954) also investigated the acute toxicity of DiBP (purity not reported) following i.p. injection in rats (albino), mice (young adults), rabbits, guinea pigs, and cats (strains not reported). As described above, multiple doses were administered to groups of 2–3 rats or mice, and LD_{50} values were determined based on 24-hour mortalities. Dose groups of rabbits, guinea pigs, and cats comprised only a single animal each. Mortality was observed at lower doses when administered intraperitoneally rather than orally. The i.p. LD_{50} values were approximately 7,300 and 9,300 mg/kg in rats and mice, respectively. The highest doses given by i.p. injection that did not result in mortality during the 24 hours following dosing were approximately 9,300, 4,100, and 1,000 mg/kg in rabbits, guinea pigs, and cats, respectively. Late deaths (after 24 hours) were observed in nearly all species. Histological examination of liver and kidney sections removed from guinea pigs, rabbits, and cats surviving the 2-week observation period revealed occasional minor pathological changes described by the study authors as comparable to historical controls. Based on the single dose studies conducted by Hodge (1954), DiBP was slightly more acutely toxic in guinea pigs and cats when administered intraperitoneally than in rabbits, mice, and rats.

5.5. Primary Skin Irritation

Lawrence et al. (1975, 1971) reported that DiBP did not act as a primary irritant in New Zealand rabbits following intradermal injection, but few details were provided. Unpublished data compiled by the European Commission (2004, 2000) also suggest that DiBP is not a skin irritant in rabbits. Eastman Kodak Co. (1978) indicated that DiBP is a slight skin irritant in guinea pigs based on unpublished data, but no further information was provided.

The lack of additional methodological information and presentation of disparate results on the irritant properties of DiBP can be considered a data gap and supports the conclusion that there is "inadequate evidence" for the designation of DiBP as a dermal "primary irritant" when considering FHSA criteria (16 CFR §1500.3(c)(4)).

A weight of evidence supports the conclusion that DiBP does not fit the definition of a dermal "corrosive" as outlined in the FHSA (16 CFR §1500.3(c)(3)).

5.6. Primary Eye Irritation

DiBP was not irritating to the eye in rabbits following ocular instillation, but few details were provided (Lawrence et al., 1975, 1971). Unpublished data compiled by the European Commission (2004, 2000) also suggest that DiBP is not an eye irritant in rabbits.

The weight of evidence including sufficient animal data supported the conclusion that DiBP did not fit the definition of an ocular "corrosive" as outlined in the FHSA (16 CFR §1500.3(c)(4)).

The lack of additional methodological information on the ocular irritant properties of DiBP can be considered a data gap and supports the conclusion that there is "inadequate evidence" for the designation of DiBP as an ocular "primary irritant" when considering FHSA criteria (16 CFR §1500.3(c)(3)).

5.7. Sensitization

Eastman Kodak Co. (1978) indicated that DiBP is not a skin sensitizer in guinea pigs based on unpublished data, but no further information was provided.

The lack of additional methodological information on the sensitization properties of DiBP can be considered a data gap and supports the conclusion that there is "inadequate evidence" for the designation of DiBP as a dermal "strong sensitizer" as defined in the FHSA (16 CFR §1500.3(c)(5)).

REPEATED DOSE TOXICITY

5.8. General Effects (Clinical Signs, Food/Water Consumption, Body Weight)

No clinical signs or significant effects on food consumption or body weight were observed among rats treated orally with DiBP following short-term exposures (1–2 weeks) (BUA, 1998, as cited by the European Commission, 2004, 2000; Oishi and Hiraga, 1980a). However, rats fed a diet containing the monoester, MIBP, at 1,083 mg/kg-day for 7 days exhibited suppressed food consumption throughout the experimental period and a significant reduction in body weight (Oishi and Hiraga, 1980b). In mice, short-term oral studies have reported conflicting effects of DiBP exposure on body weight gain, although this may be a consequence of experimental differences between studies, including strain of mouse used and method of administration. In 7-day feeding studies, dietary exposures to 2,083 mg DiBP/kg-day or 2,083 mg MIBP/kg-day caused notable suppression in feed consumption and significant reductions in body weight among JCL:ICR mice (Oishi and Hiraga, 1980c, d). No significant effects on body weight were observed among CD-1 mice in an 8-day gavage study at doses as high as 10,400 mg/kg-day (Hardin et al, 1987; NIOSH, 1983), indicating that dietary studies showing suppression of food consumption and body weight following exposure to DiBP may have been reflecting a palatability issue rather than a true toxic effect. However, clinical signs including languidness, abdominal and anal urine stains, and rough hair coat were reported in the gavage study at doses ≥3,225 mg/kg-day.

Rats fed diets containing DiBP at the 5% level for 1 or 4 months exhibited significant reductions in body weight compared to controls and/or low-dose groups (Hodge, 1954, 1953). No effects on body weight were seen at dietary levels of ≤1–2%. The data are shown in Table 5.2. No significant effects on survival were observed in these studies, and effects on clinical signs and food consumption were not assessed.

Table 5.2. Body Weight Data for Weanling Albino Rats Exposed to DiBP in the Diet for 1 or 4 Months

Endpoint	DiBP Concentration in Diet					
	0	**0.01%**	**0.1%**	**1.0%**	**2.0%**	**5.0%**
1-Month study (males only)						
Estimated dose (mg/kg-day)	0	15	142	1,417	2,975	8,911
Body weight (g)	226 ± 25[a,b]	189 ± 15	194 ± 26	189 ± 15	181 ± 24	141 ± 14[c]
4-Month study (males)						
Estimated dose (mg/kg-day)	0	NT	67	738	NT	5,960
Body weight (g)	359 ± 37	NT	373 ± 48.1	326 ± 33.4	NT	204 ± 24.1[d]
4-Month study (females)						
Estimated dose (mg/kg-day)	0	NT	85	795	NT	4,861
Body weight (g)	216 ± 16.5	NT	214 ± 25.7	231 ± 24.3	NT	187 ± 15.5[d]

[a]Values are means ± SD.
[b]SDs were calculated for this review from individual animal data for this study.
[c]Significantly different from low-dose group, $p < 0.05$ (unpaired t-test conducted for this review).
[d]Significantly different from control group, $p < 0.05$ (unpaired t-test conducted for this review).

NT = not tested

Source: Hodge (1954, 1953).

Two dogs fed diets containing DiBP for 2 months (a male treated with 1 mg/kg-day and a female treated with 16 mg/kg-day) remained in good general condition throughout the test, with both animals maintaining healthy appetites, exhibiting no signs of illness, and showing only minor changes in weight (the male dog gained 1 pound and the female dog lost 0.5 pounds) (Hodge, 1954). Based on unpublished data summarized by the European Commission, survival was unaffected, but food intake and body weight were decreased, and diarrhea and emesis were observed, in four cats administered DiBP via gavage for 3 months at 2,080 mg/kg-day (BASF, 1961, as cited by the European Commission, 2004, 2000). No further information was provided.

No significant treatment-related effects were observed based on survival or clinical signs among pregnant rats treated orally with DiBP during gestation at doses up to 900 mg/kg-day (Boberg et al., 2008; Howdeshell et al., 2008; Saillenfait et al., 2008, 2006, 2005; Borch et al., 2006). However, several studies have reported depressed gestational weight gain among rats treated orally with DiBP during gestation at doses as low as 500 mg/kg-day (Howdeshell et al., 2008; Saillenfait et al., 2006, 2005; BASF, 2003, as cited by the European Commission, 2004). Among these studies, BASF (2003) was the only one to report corresponding suppression of food consumption in exposed dams. Data summarized by the European Commission (2004,

2000) for this study also indicated that the effect on body weight gain was maintained even after adjusting for gravid uterine weight. In contrast, the studies by Saillenfait et al. (2006, 2005) found that significant differences in gestational weight gains were no longer observed following adjustment for gravid uterine weights. Other studies in rats found no significant effects on maternal body weight gain following oral exposure to DiBP (Boberg et al., 2008; Saillenfait et al., 2008; Borch et al., 2006).

A single study is available for gestational exposure to DiBP in mice (Hardin et al., 1987; NIOSH, 1983). Severe maternal mortality (27/50 maternal mice died) and no successful deliveries resulted from gavage treatment on gestation days (GDs) 6–13 with a high dose of DiBP (4,000 mg/kg-day). This study identified 4,000 mg/kg-day as a lethal dose for pregnant mice.

5.9. Hematology

No significant treatment-related changes in hematology were reported among rats treated orally with DiBP for 4 months at doses up to 5,960 mg/kg-day; Hodge, 1954). Occasional elevations in white blood cell (WBC) counts were observed in these rats, but these changes were attributed to the presence of infection in the colony; a number of cases of "sniffles" were reported. The only other hematological data available are unpublished data summarized by the European Commission reporting that blood parameters were unchanged in cats administered DiBP at 2,080 mg/kg-day via gavage for 3 months (BASF, 1961, as cited by the European Commission, 2004, 2000). No further details were available.

5.10. Hepatotoxicity

Short-term oral exposure (1–2 weeks) to DiBP has been shown to cause a significant increase in liver weights among both rats and mice at doses of 1,212 mg/kg-day (BUA, 1998, as cited by the European Commission, 2004, 2000; Oishi and Hiraga, 1980a, c). Corresponding changes in clinical chemistry associated with liver effects reported in these studies include decreased liver zinc levels, increased serum albumin levels, and decreased serum triglyceride and cholesterol levels (BUA, 1998, as cited by the European Commission, 2004, 2000; Oishi and Hiraga, 1980a, c). Dietary exposure to the monoester, MIBP, at 2,083 mg/kg-day also caused a significant increase in liver weights among mice (Oishi and Hiraga, 1980d).

Signs of peroxisome proliferation were also reported in hepatic tissues of rats following gavage treatment with DiBP at doses ≥100 mg/kg-day, but data from this study are available only from a secondary source, precluding identification of reliable effect levels (BUA, 1998, as cited by the European Commission, 2004, 2000).

Subchronic (1–4 months) oral exposure to DiBP caused significant increases in liver weights among rats at dietary concentrations of 1% (Hodge, 1954, 1953). In these studies, liver weight was unaffected at ≤ 0.1% (Table 5.3). Pathological examinations of liver tissue were reported to be unremarkable. A female dog fed a diet with DiBP for 2 months had an enlarged liver without corresponding histological changes (Hodge, 1954), but limitations of this study (only two animals tested and no corresponding controls) preclude derivation of effect levels for hepatotoxicity. Similarly, although unpublished data summarized by the European Commission indicated no effects of gavage treatment with DiBP on liver function in cats (BASF, 1961, as cited by the European Commission, 2004, 2000), the lack of quantitative information limits the interpretation of these results.

Table 5.3. Liver Weight Data for Weanling Albino Rats Exposed to DiBP in the Diet for 1 or 4 Months

Endpoint	DiBP Concentration in Diet					
	0	**0.01%**	**0.1%**	**1.0%**	**2.0%**	**5.0%**
1-Month study (males only)						
Estimated dose (mg/kg-day)	0	15	142	1,417	2,975	8,911
Absolute liver weight (g)	10.2 ± 0.7[a,b]	8.44 ± 0.7	8.93 ± 1.3	10.7 ± 1.1[c]	11.7 ± 2.1[c]	11.5 ± 1.3[c]
Relative liver weight (mg/g-body weight)	45.2 ± 2.8	44.7 ± 2.8	47.3 ± 13	56.7 ± 1.7[d]	64.6 ± 4.4[d]	80.9 ± 1.2[d]
4-Month study (males)						
Estimated dose (mg/kg-day)	0	NT	67	738	NT	5,960
Absolute liver weight (g)	11.7 ± 1.3	NT	12.4 ± 1.6	13.1 ± 2.3	NT	12.3 ± 1.8
Relative liver weight (mg/g-body weight)	32.6 ± 2.0	NT	33.2 ± 0.55	39.9 ± 4.2[d]	NT	60.1 ± 3.2[d]
4-Month study (females)						
Estimated dose (mg/kg-day)	0	NT	85	795	NT	4,861
Absolute liver weight (g)	7.5 ± 0.61	NT	7.5 ± 0.84	8.6 ± 0.82	NT	10.5 ± 1.2[d]
Relative liver weight (mg/g-body weight)	34.8 ± 3.0	NT	34.9 ± 1.8	37.5 ± 3.4	NT	56.4 ± 2.0[d]

[a]Values are means ± SD.
[b]SDs were calculated for this review from individual animal data for this study.
[c]Significantly different from low-dose group, $p < 0.05$ (unpaired t-test conducted for this review).
[d]Significantly different from control group, $p < 0.05$ (unpaired t-test conducted for this review).

NT = not tested

Source: Hodge (1954, 1953).

The weight of evidence from the above studies supported the conclusion that there was "sufficient animal evidence" for the designation of DiBP as a "hepatotoxicant".

5.11. Renal Toxicity

There was a small, statistically significant decrease in relative kidney weight in mice treated with 2,083 mg/kg-day of DiBP in the diet for 1 week (Oishi and Hiraga, 1980c). However, short-term exposure to MIBP resulted in only slight, nonsignificant decreases in kidney weights among treated mice (Oishi and Hiraga, 1980d), and no significant changes in kidney weights were reported in short-term studies of DiBP in rats (BUA, 1998, as cited by the European Commission, 2004, 2000; Oishi and Hiraga, 1980a). Dietary exposure of rats to DiBP at ≥2% for 1 month resulted in significant increases in relative kidney weights, but with no changes in absolute kidney weights and no corresponding histological changes (Hodge, 1953).

Dietary exposure of rats to DiBP at up to 5% for 4 months did not result in any significant changes in the kidneys (Hodge, 1954).

5.12. Reproductive Toxicity

No 1- or 2-generation reproductive toxicity tests of DiBP exposure are available in animals or humans. However, repeated-dose administration of DiBP has been shown to induce adverse effects on reproductive parameters (e.g., testes weights, testosterone levels, spermatogenesis) in adolescent and adult male rodents.

Short-term oral exposure to DiBP causes significant adverse testicular effects in male adolescent rats including decreased testes weights, increased numbers of apoptotic spermatogenic cells, disorganized or reduced vimentin filaments in Sertoli cells, elevated testicular testosterone levels, decreased testicular zinc levels, and marked inhibition of spermatogenesis and desquamation of spermatocytes. Effects were seen at doses as low as 500 mg/kg-day (Zhu et al., 2010; Oishi and Hiraga, 1980a). Similar findings were reported in rats treated with MIBP (Foster et al., 1981; Oishi and Hiraga, 1980b). Studies in mice found different results. In the Zhu et al. (2010) study, mice treated with DiBP for 7 consecutive days only exhibited a significant decrease ($p < 0.01$) in testes weight at 1,000 mg/kg-day and no effect on numbers of apoptotic spermatogenic cells. In the Oishi and Hiraga (1980c) study, there was an increase in relative testes weight (possibly secondary to reduced body weight in these animals; absolute testes weights were not reported) and no effect on testicular testosterone levels at 2,083 mg/kg-day DiBP. Testicular zinc concentrations were reduced as in rats, however (Oishi and Hiraga, 1980c). MIBP produced similar results to DiBP for testes weight and zinc concentration in mice, but in contrast to the results for DiBP in mice and both DiBP and MIBP in rats, produced a large significant reduction in testicular testosterone in this species (Oishi and Hiraga, 1980d).

Subchronic oral exposure to DiBP resulted in marked significant reductions in absolute and relative testes weights of adult male rats fed 5% in the diet for 4 months (Hodge, 1954). There was no difference from controls at 1% in the diet. The data are shown in Table 5.4. No histopathology was conducted in this study. The only other repeated-dose subchronic data available that evaluated effects on the male reproductive tract come from a limited dog study conducted by the same researchers (Hodge, 1954). Histopathological examination of the testes from the one male dog tested in this study revealed very few mature sperm, but no other significant testicular changes were reported.

Table 5.4. Testes Weight Data for Male Albino Rats Exposed to DiBP in the Diet for 4 Months

Endpoint	DiBP Concentration in Diet			
	0	**0.1%**	**1.0%**	**5.0%**
Estimated dose (mg/kg-day)	0	67	738	5,960
Absolute testes weight (g)	3.06 ± 0.28[a,b]	3.11 ± 0.28	3.02 ± 0.17	0.93 ± 0.11[c]
Relative testes weight (mg/g-body weight)	8.33 ± 1.16	8.40 ± 0.87	9.29 ± 0.64	4.58 ± 0.58[c]

[a]Values are means ± SD.
[b]SDs were calculated for this review from individual animal data for this study.
[c]Significantly different from controls, $p < 0.05$ (unpaired t-test conducted for this review).

Source: Hodge (1954).

The weight of evidence from the above studies supported the conclusion that there was "sufficient animal evidence" for the designation of DiBP as a "reproductive toxicant".

5.13. Prenatal, Perinatal and Post-natal Toxicity

In humans, biological monitoring data have shown a correlation between the concentrations of MIBP in the urine of pregnant women and reduced anogenital distance (AGD) and anogenital index (AGI, AGI = AGD/body weight) in male infants (Marsee et al., 2006; Swan et al., 2006, 2005). Study limitations such as the estimation of exposure using only a single urine sample from each woman obtained fairly late during pregnancy, analysis of AGD based on only a single measurement in boys of differing ages, and small study size limit the interpretation of these results. Reduced play behavior in preschool-aged boys has also been correlated with MIBP levels in maternal urine (Swan et al., 2010). However, similar limitations as the earlier study, including an inadequate number of maternal urine samples and small study size, increase the uncertainties associated with this study.

In animals, the developmental toxicity of DiBP has been evaluated in several studies of pregnant rats exposed orally during gestation. These studies have reported that DiBP exposure in utero results in significant adverse effects to the offspring of the exposed dams.

Saillenfait and colleagues investigated the developmental toxic effects of DiBP in a series of studies in Sprague-Dawley rats (Saillenfait et al., 2008, 2006, 2005). In a preliminary study, DiBP (in olive oil, purity not reported) was administered via gavage to groups of 10–14 timed-

pregnant Sprague-Dawley rats at 0, 250, 500, 750, or 1,000 mg/kg-day on GDs 6–20 (Saillenfait et al., 2005). There was a significant dose-related increase in the incidence of resorptions at ≥500 mg/kg-day and significant dose-related decreases in number of live fetuses per litter and fetal body weight at ≥750 mg/kg-day (Table 5.5). Internal examination revealed undescended testes in 56 and 70% of the male fetuses at 750 and 1,000 mg/kg-day, respectively; undescended testes were not found in fetuses in the control or lower dose groups. No significant difference in fetal sex ratio was observed. These results indicate developmental no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) values of 250 and 500 mg/kg-day, respectively, for increased incidence of resorptions. Testicular effects in male fetuses (increased incidence of litters with fetuses with undescended testes) occurred at higher doses.

Table 5.5. Litter Effects in Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 6–20

Endpoint	Dose (mg/kg-day)				
	0	250	500	750	1,000
Number of implantations/litter	13.5 ± 2.1[a]	13.4 ± 2.9	14.0 ± 3.6	13.0 ± 3.0	12.6 ± 5.5
Percentage of resorptions/litter	2.5 ± 8.2	4.2 ± 6.9	8.8 ± 10.4[b]	38.3 ± 34.4[b]	61.2 ± 31.2[b]
Number of live fetuses/litter	13.2 ± 2.6	12.8 ± 2.9	12.6 ± 3.5	8.1 ± 5.2[b]	5.7 ± 5.5[b]
Fetal body weight (g)	5.6 ± 0.1	5.8 ± 0.3	5.4 ± 0.4	4.7 ± 0.6[b]	4.4 ± 0.6[b]
Percentage of male fetus/litter	47.4 ± 14.0	51.3 ± 19.7	50.5 ± 15.2	45.6 ± 21.0	58.6 ± 25.3
Incidence of male fetuses (litters) with undescended testes	0/70 (0/11)	0/68 (0/10)	0/68 (0/11)	31/55 (9/14)	26/37 (7/12)

[a]Values are means ± SD.
[b]Significantly different from controls, $p < 0.05$.

Source: Saillenfait et al. (2005).

Saillenfait et al. (2006) exposed groups of 20–22 timed-pregnant Sprague-Dawley rats to DiBP (≥99% pure, in olive oil) via gavage doses of 0, 250, 500, 750, or 1,000 mg/kg-day on GDs 6–20. As in the preliminary study, there was a marked increase in resorptions, a significant reduction in the number of live fetuses per litter, and a significant decrease in fetal body weight at ≥500 mg/kg-day (Table 5.6). In addition, the incidences of external, visceral, and skeletal malformations were increased after exposure to ≥750 mg/kg-day (Table 5.7). Aside from a significant increase in the incidence of fused sternebrae, there were no significant differences in the incidence of any specific malformations when considered individually. However, the incidences based on total number of fetuses with external malformations and total numbers of fetuses or litters with visceral or skeletal malformations were significantly higher than controls.

The most common malformations observed were anterior neural tube closure defects, anophthalmia, urinary tract and vascular defects, and defects of the axial skeleton, including fused vertebral arch or centrum, hemicentrum, and sternal abnormalities. Visceral and skeletal variations were increased as well. There were significant increases in the incidences of undescended testes and supernumary ribs in groups exposed to ≥750 mg/kg-day. Additionally, in groups exposed to ≥500 mg/kg-day, the degree of transabdominal testicular migration (TTM) in relation to the bladder was significantly increased over that of controls. An increasing value of TTM indicates decreased descendence of the testes during development. In fact, at the highest dose, about two-thirds of the testes were located in the upper half of the abdominal cavity. This study identified a LOAEL of 500 mg/kg-day and a NOAEL of 250 mg/kg-day for developmental toxicity based on reduced fetal growth and increased degree of TTM. Male fetuses with ectopic (undescended) testis appeared at the LOAEL, and their incidence increased with dose. Increased incidences of resorptions and developmental malformations were seen at higher doses (≥750 mg/kg-day).

Table 5.6. Implantation, Fetal Survival, and Fetal Body Weight Data for Pregnant Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 6–20

Endpoint	Dose (mg/kg-day)				
	0	**250**	**500**	**750**	**1,000**
Number of live litters[a]	22/22	21/22	21/22	21/21	18/20
Number of implantation sites/litter[b]	13.6 ± 2.0	13.6 ± 2.9	12.1 ± 4.5	14.2 ± 2.4	13.5 ± 2.9
Percentage of postimplantation loss/litter[b]	6.7 ± 7.6	11.0 ± 23.6	13.9 ± 20.9	28.2 ± 18.9[c]	59.6 ± 21.5[c]
Percentage of dead fetuses/litter[b]	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 1.5	0.7 ± 2.1	0.3 ± 1.5
Percentage of resorptions/litter[b]	6.7 ± 7.6	11.0 ± 23.6	13.6 ± 20.8	27.6 ± 18.2[c]	59.3 ± 22.2[c]
Number of live fetuses/litter[b]	12.8 ± 2.4	13.1 ± 3.1	11.3 ± 3.5	10.1 ± 3.1[c]	6.2 ± 3.2[c]
Percentage of male fetuses/litter[b]	52.4 ± 14.0	50.5 ± 11.1	49.6 ± 19.4	52.6 ± 16.7	61.5 ± 19.0
Fetal body weight (g)					
All[b]	5.71 ± 0.28	5.69 ± 0.33	5.31 ± 0.40[c]	4.72 ± 0.33[c]	4.32 ± 0.35[c]
Male[b]	5.84 ± 0.31	5.85 ± 0.34	5.49 ± 0.48[c]	4.85 ± 0.32[c]	4.39 ± 0.43[c]
Female[b]	5.57 ± 0.29	5.52 ± 0.34	5.10 ± 0.33[c]	4.57 ± 0.4[c]	4.14 ± 0.35[c]

[a]Live litters/total litters including pregnant females at euthanization.
[b]Values are means ± SD.
[c]Significantly different from controls mean, $p < 0.05$.

Source: Saillenfait et al. (2006).

Table 5.7. Malformations and Variations in Fetuses of Pregnant Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 6–20

Endpoint	Dose (mg/kg-day) 0	250	500	750	1,000
Number of fetuses (litters) examined					
External	281 (22)	276 (21)	237 (21)	212 (21)	111 (18)
Visceral	141 (22)	138 (21)	119 (21)	106 (21)	56 (18)
Skeletal	140 (22)	138 (22)	118 (22)	106 (21)	55 (18)
Malformations					
Number of fetuses (%) with malformations					
External[a]	0	0	0	5 (2.4)[d]	6 (5.4)[d]
Visceral[b]	0	2 (1.4)	2 (1.7)	13 (12.3)[d]	10 (17.9)[d]
Skeletal[c]	0	0	4 (3.4)	18 (17.0)[d]	34 (61.8)[d]
Number of litters (%) with malformations					
External[a]	0	0	0	4 (19.0)	4 (22.2)
Visceral[b]	0	1 (4.8)	2 (9.5)	8 (38.1)[d]	8 (44.4)[d]
Skeletal[c]	0	0	4 (19.0)	11 (52.4)[d]	15 (83.3)[d]
Mean % fetuses with malformations/litter					
External[a]	0	0	0	2.3 ± 5.1	3.7 ± 7.7
Visceral[b]	0	1.2 ± 5.5	1.7 ± 5.5	13.1 ± 23.9[e]	15.8 ± 20.9[e]
Skeletal[c]	0	0	3.2 ± 6.9	18.3 ± 22.8[e]	67.1 ± 37.1[e]
Number of fetuses (litters) with fused or fused and scrambled sternebrae	0	0	0	12 (7)[e]	26 (13)[e]
Variations					
Number of fetuses (litters) with visceral variations					
Ectopic testes[f]	0	0	3 (2)	30 (16)[e]	30 (16)[e]
TTM[g]	2.6 ± 3.6	3.8 ± 3.3	13.6 ± 11.0[e]	42.2 ± 11.8[e]	58.1 ± 12.8[e]
Number of fetuses (litters) with skeletal variations					
Sternebrae, fused first and second	1 (1)	0	8 (4)	29 (11)[e]	5 (4)
Ribs					
Cervical, rudimentary	0	0	2 (2)	12 (9)[e]	9 (6)
14th, any supernumerary	23 (11)	32 (14)	42 (18)	72 (20)[e]	52 (18)[e]
14th, long supernumerary	1 (1)	1 (1)	2 (2)	15 (9)[e]	9 (9)[e]
Thoracic or lumbar vertebral centra, incomplete ossification	3 (2)	8 (6)	7 (7)	18 (14)[e]	16 (8)[e]

[a]Incidences of fetuses or litters with individual external malformations in exposed groups were not significantly ($p < 0.05$) different from control values. External malformations observed in more than one fetus were exencephaly and meningoencephalocele.
[b]Incidences of fetuses or litters with individual visceral malformations in exposed groups were not significantly ($p < 0.05$) different from control values. Visceral malformations observed in more than one fetus were anophthalmia, transposed aorta or pulmonary artery, and absent kidney and ureter.
[c]Incidences of fetuses or litters with individual skeletal malformations in exposed groups were not significantly ($p < 0.05$) different from control values, except for incidences of fetuses or litters with fused or fused and scrambled sternebrae. Other skeletal malformations observed in more than one fetus were cleft sternum, fused ribs, fused cervical archs, fused or misaligned thoracic or lumbar centra, and hemicentric thoracic or lumbar centrum.
[d]Significant difference from the vehicle control, $p < 0.05$ (Fisher's test).
[e]Significant difference from the vehicle control, $p < 0.05$ (Mann-Whitney test).
[f]Testes outside the normal pathway of descent.
[g]Degree of TTM: (Distance between the bladder neck and the lower pole of the testis) / (distance between the lower pole of the kidney and the bladder neck) × 100. The value of TTM increases with degree of inhibition of normal descent of testes during development.

Source: Saillenfait et al. (2006).

Saillenfait et al. (2008) conducted a follow-up study to further evaluate the postnatal effects of in utero exposure to DiBP on male reproductive development. DiBP (>99% pure in olive oil) was administered via gavage to groups of 11–13 timed-pregnant Sprague-Dawley rats at 0, 125, 250, 500, or 625 mg/kg-day on GDs 12–21. This period of gestation is considered a sensitive time for male reproductive tract differentiation in rats. Litters were born and pups observed through postnatal weeks (PNWs) 16–17 in some cases. In contrast to the previous studies with dosing on GDs 6–20, there was no effect on postimplantation loss or number of live pups per litter. Effects observed in male offspring were reduced AGD on postnatal day (PND) 1, decreased pup weight on PND 1 and at weaning (PND 21), delayed separation of the prepuce from the glans penis, increased thoracic areolas and/or nipples, decreased testes and epididymis weights, increased incidence of testicular tubular degeneration-atrophy/hypoplasia, and increased incidence of external malformations, including hypospadias, exposed os penis, nonscrotal testes, and azospermia (Tables 5.8 and 5.9). The most sensitive of these effects (decreased male AGD at PND 1, increased thoracic areolas and/or nipples in male offspring, and increased incidence of male offspring with testicular tubular degeneration-atrophy/hypoplasia) occurred at 250 mg/kg-day, which is identified as the LOAEL. The NOAEL was 125 mg/kg-day.

Table 5.8. Birth Weight, Postnatal Survival, and Early Reproductive Organ Development Endpoints in Male Offspring of Pregnant Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 12–21

Endpoint	Dose (mg/kg-day)				
	0	125	250	500	625
Pup survival PNDs 1–4 (%)	99.4 ± 2.3[a]	98.9 ± 3.8	98.9 ± 2.7	98.2 ± 6.1	92.5 ± 12.0
Pup survival PNDs 4–21 (%)	92.5 ± 8.7	94.0 ± 7.0	97.1 ± 4.7	95.0 ± 9.0	97.0 ± 4.8
Male pup weight PND 1 (g)	7.19 ± 0.71	7.10 ± 0.70	7.04 ± 0.43	7.03 ± 0.53	6.45 ± 0.60[b]
Male AGD PND 1 (mm)	2.55 ± 0.17	2.44 ± 0.15	2.28 ± 0.30[b]	2.02 ± 0.13[b]	1.98 ± 0.16[b]
PPS in male offspring[c]					
Number of males examined (litters)	46 (12)	40 (10)	55 (14)	39 (11)	17 (7)
Age at PPS (d)[d]	46.9 ± 1.5	45.1 ± 1.6[b]	46.3 ± 1.8	51.5 ± 3.1[b]	49.8 ± 3.2[b]
Body weight at PPS (g)	215 ± 11	197 ± 15[b]	205 ± 9b	230 ± 22	220 ± 19

[a]Values are mean ± SD.
[b]Significantly different from controls, $p < 0.05$ (Mann-Whitney test).
[c]PPS describes the separation of the prepuce from the glans penis.
[d]PPS was not evaluated in males with hypospadias (i.e., 5/4 and 22/9 males/litters at 500 mg DiBP/kg-day and 625 mg DiBP/kg-day, respectively).

PPS = preputial separation

Source: Saillenfait et al. (2008).

Table 5.9. Body and Reproductive Organ Weights and Reproductive Organ Lesions of Adult Male Offspring of Pregnant Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 12–21

Endpoint	Dose (mg/kg-day)				
	0	**125**	**250**	**500**	**625**
Weights (g) at PNWs 16–17					
Number of males (litters)	22 (12)	20 (10)	27 (14)	22 (11)	18 (10)
Body weight[a]	476 ± 58	449 ± 53	452 ± 43	424 ± 44[b]	423 ± 58[b]
Right testis[a]	1.93 ± 0.16	1.80 ± 0.48	1.88 ± 0.28	1.62 ± 0.62[c]	0.98 ± 0.76[b,c]
Right epididymis[a]	0.64 ± 0.06	0.59 ± 0.10	0.60 ± 0.06	0.45 ± 0.18[b]	0.36 ± 0.18[b]
Left testis[a]	1.93 ± 0.17	1.76 ± 0.44	1.90 ± 0.28	1.84 ± 0.31[c]	1.17 ± 0.83[b,c]
Left epididymis[a]	0.63 ± 0.06	0.58 ± 0.10	0.60 ± 0.06	0.52 ± 0.13[b]	0.37 ± 0.16[b]
Seminal vesicles[a]	1.90 ± 0.29	1.86 ± 0.27	1.76 ± 0.20	1.45 ± 0.25[b]	1.27 ± 0.41[b]
Prostate[a]	1.10 ± 0.23	0.95 ± 0.20[b]	1.0 ± 0.13	0.91 ± 0.13[b]	0.79 ± 0.23[b]
Thoracic areolas/nipples in male offspring (incidence)					
PNDs 12–14	0/76	0/78	8/96	47/79	56/76
PNWs 11–12 and 16–17	0/46	0/40	4/55	24/44	29/38
External malformations at PNWs 11–12 and 16–17					
Number of adults examined	46	40	55	44	39
Hypospadias	0	0	0	5	22
Exposed os penis	0	0	0	4	11
Cleft prepuce	0	0	0	0	10
Nonscrotal testes	0	0	0	11	30
Histopathology (PNWs 11-12)					
Number males/litters examned[d]	24/12	20/10[e]	28/14[e]	22/11	20/10
Epididymides:					
Oligospermia	0	1	3	2[f]	1[f]
Azoospermia	0	1	3	10[f]	18[f]
Granulomatous inflammation	0	0	0	4	3
Testes: tubular degeneration-atrophy/hypoplasia[g]	2	2	7	16	20
Grade 1 (<5% tubules affected)	2	0	1	3	1
Grade 2 (5–25%)	0	1	1	1	0
Grade 3 (26–45%)	0	0	2	0	2
Grade 4 (46–85%)	0	0	1	4[h]	0
Grade 5 (>85%)	0	1	2	8[h]	17
Testes: tubular necrosis	0	0	1	3	5
Testes: interstitial cell hyperplasia	0	0	0	1	9

[a]Values are mean ± SD. Severely underdeveloped testis and/or epididymis were not included in organ weight means.
[b]Significantly different from controls, $p < 0.05$ (Mann-Whitney test).
[c]When only descended testes were included, the means at 500 mg DiBP/kg-day are 1.77 ± 0.46 (right, n=17) and 1.90 ± 0.16 (left, n=19), and the means at 625 mg DiBP/kg-day are 1.83 ± 0.48 (right, n=7) and 1.87 ± 0.46 (left, n=8); no nonscrotal testis in control.
[d]Two males in each litter.
[e]Histological examination was also performed on two 125 mg DiBP/kg-day males and one 250 mg DiBP/kg-day male, which showed small testes (bilateral) at necropsy on PNWs 16–17. All three had severe degeneration of seminiferous tubules (grade 5), together with oligospermia or azoospermia. These data are not included in the table. At 125 mg DiBP/kg-day, all affected rats were from the same litter.
[f]One male at 500 mg DiBP/kg-day and three males at 625 mg DiBP/kg-day showed azoospermia in one epididymis and oligospermia in the other one. Only azoospermia is mentioned.

[g]Only the highest severity of tubular degeneration-atrophy/hypoplasia was mentioned when the lesion was bilateral; severity based on the approximate percentage of affected semiferous tubules.
[h]In this dose group, 6 of the 12 males with grade 4 or 5 tubular degeneration had descended testes.

Source: Saillenfait et al. (2008).

In another study, in which groups of 5–8 pregnant Sprague-Dawley rats were given DiBP (99% pure, in corn oil) via gavage doses of 0, 100, 300, 600, or 900 mg/kg-day on GDs 8–18, Howdeshell et al. (2008) found that fetal testicular testosterone production was significantly reduced in a dose-related manner at ≥300 mg/kg-day (Table 5.10). Effects at higher doses were increased resorptions and decreased number of liver fetuses per litter, as in the first two Saillenfait et al. (2006, 2005) studies. These results indicate developmental NOAEL and LOAEL values of 100 and 300 mg/kg-day, respectively, for decreased fetal testicular testosterone production.

Table 5.10. Fetal Effects in Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 8–18

Endpoint	Dose (mg/kg-day)				
	0	100	300	600	900
Number of pregnant females on GD 18	5/5[a] (100%)	8/8 (100%)	5/8 (63%)	5/5 (100%)	5/5 (100%)
Number of implantations[b,c]	13.7 ± 0.9[d] (3)	14.8 ± 0.8 (4)	16.0 ± 1.0 (3)	12.7 ± 1.2 (3)	13.3 ± 0.9
Number of live fetuses[c]	13.3 ± 0.7 (3)	13.5 ± 0.5 (4)	15.3 ± 1.5 (3)	9.3 ± 2.6 (3)	5.0 ± 3.6[e] (3)
Total resorptions	0.2 ± 0.2	1.0 ± 0.5	0.4 ± 0.4	2.0 ± 1.1	7.8 ± 2.5[e]
Fetal mortality (%)[c,f]	1.3 ± 1.3 (3)	4.6 ± 2.6 (4)	2.7 ± 2.7 (3)	17.2 ± 10.4	59.0 ± 30.2[e]
Testicular testosterone production on GD 18 (ng/testis/3 hour)[g]	5.7 ± 0.13 (15/5)	5.44 ± 0.19 (24/8)	3.40 ± 0.28[e] (15/5)	2.31 ± 0.35[e] (15/5)	2.09 ± 0.91[e] (6/2)

[a]Number observed/total tested.
[b]Implantations = live fetuses + dead fetuses + total resorptions.
[c]Number in parentheses indicates number of litters in the analysis when different from number of dams on GD 18.
[d]Values are means ± standard error (SE).
[e]Significantly different from controls, $p < 0.05$.
[f]Fetal mortality = ([resorptions + dead fetuses] / implantations) × 100.
[g]Numbers in parentheses indicate number of individual fetuses examined / number of litters examined.

Source: Howdeshell et al. (2008).

In another evaluation of the ability of DiBP to interfere with male reproductive tract development in rats, groups of eight timed-pregnant Wistar rats (HanTac:WH) were given DiBP (99% pure, in corn oil) via gavage doses of 0 or 600 mg/kg-day from GD 7 to the time of autopsy on GD 19 or 20/21 (Boberg et al., 2008; Borch et al., 2006). There were no effects on

resorptions or fetal viability in this study. Observed effects in male fetuses were consistent with the results of other studies: decreased fetal body weight, reduced AGD (in males; increased AGD in females), significantly reduced testicular testosterone content and testicular testosterone production ex vivo, impaired testicular development indicated by clustering of small Leydig cells and Sertoli cell vacuolization (Table 5.11), and decreased plasma levels of insulin and leptin and decreased testicular mRNA levels for *insl-3*, several steroid synthesis genes, and PPARα. Based on these effects, the dose level of 600 mg/kg-day was a LOAEL in this study.

Table 5.11. Testicular Histopathology in Male Rat Fetuses Exposed to DiBP on GDs 7–19 or 20/21

Histology[a]	GD 19		GD 20/21	
	Control	600 mg/kg-day	Control	600 mg/kg-day
Clustering of small Leydig cells	2/13	9/9[b]	0/10	13/15[b]
Sertoli cells vacuolization	0/13	1/9	0/10	14/16[b]
Central localization of genocytes	0/13	2/9	0/10	14/16[b]
Multinuclear genocytes	1/13	0/9	1/10	10/16[c]

[a]One or two testes per litter were examined microscopically.
[b]Significantly different from controls, $p < 0.001$ (Fisher's exact test).
[c]Significantly different from controls, $p < 0.05$ (Fisher's exact test).

Source: Borch et al. (2006).

An unpublished study submitted by BASF (2003) to the European Commission (2004), in which DiBP (purity not reported) was administered to groups of pregnant Wistar rats (25 females/group) in the diet at 0, 88, 363, or 942 mg/kg-day on GDs 6–20, reported no effect on resorptions or viable fetuses, and no significant increases in the incidence of any external, soft-tissue, or skeletal malformations. Observed effects were slightly decreased fetal body weight (about 5% below controls) and increased incidence of some skeletal variations (incomplete or unilateral ossification of sternebrae) at the high dose. The secondary source reports that this study did not provide data on male reproductive system parameters. The use of dietary rather than gavage exposure in this study may have contributed to the reduced severity of developmental effects in this study relative to the others described above.

The weight of evidence from the above studies supported the conclusion that there was "sufficient animal evidence" for the designation of DiBP as a "developmental toxicant."

5.14. Carcinogenicity

Genotoxicity

DiBP has been tested in a limited number of short-term in vitro genotoxicity assays. Consistent negative findings have been reported in bacterial assays with *Salmonella typhimurium* (Seed, 1982; Zeiger et al., 1982; Simmon et al., 1977). However, DiBP was reported to induce DNA damage in vitro in a comet assay using human cells (Kleinsasser et al., 2001a, b, 2000). These data are summarized in Table 5.12.

Table 5.12. Summary of In Vitro Genotoxicity Tests of DiBP

Assay	Result		Concentration	Reference
	Without S9	With S9		
Bacterial reverse mutation				
TA98	–	–	DiBP 100–10,000 µg/plate	Zeiger et al., 1982
TA100	–	–	DiBP 100–10,000 µg/plate	Zeiger et al., 1982
TA1535	–	–	DiBP 100–10,000 µg/plate	Zeiger et al., 1982
TA1537	–	–	DiBP 100–10,000 µg/plate	Zeiger et al., 1982
TA98	–	–	DiBP up to 5,000 µg/plate	Simmon et al., 1977
TA100	–	–	DiBP up to 5,000 µg/plate	Simmon et al., 1977
TA1535	–	–	DiBP up to 5,000 µg/plate	Simmon et al., 1977
TA1537	–	–	DiBP up to 5,000 µg/plate	Simmon et al., 1977
TA1538	–	–	DiBP up to 5,000 µg/plate	Simmon et al., 1977
8-Azaguanine resistance assay				
TA100	–	–	DiBP concentration not reported	Seed, 1982
Comet assay (DNA damage)				
Human oropharyngeal, nasal, or laryngeal mucosal cells and blood lymphocytes	+	N/A	354 µmol/mL	Kleinsasser et al., 2001a, b, 2000

Initiation and Promotion

No initiation or promotion studies were located for DiBP.

Carcinogenicity Studies

No carcinogenicity or chronic studies were located for DiBP.

The weight of evidence from the above studies supported the conclusion that there was "insufficient animal evidence" for the designation of DiBP as a "carcinogen."

6. EXPOSURE

HSDB (2009) has reported that based on monitoring and use data, the general population may be exposed to DiBP through ingestion of food and drinking water, dermal contact with products containing DiBP, and inhalation of ambient air. Occupational exposure may occur through inhalation and dermal contact where DiBP is produced or used. The European Chemicals Agency (ECHA;2009) has compiled available information on consumer exposure to DiBP. Concentrations in various consumer products, food, and other media reported by ECHA (2009) are shown in Table 6.1.

Table 6.1. Concentrations of DiBP in Various Media (ECHA, 2009)

Product/Sample	Concentration	Reference
Product		
Crayons Bar ends of run bikes, Erasers and school bags	Not reported	Stiftung Warentest, 2008a Stiftung Warentest, 2008b Danish EPA, 2007
Suckers, plastic spoons and forks, boxes for microwave ovens, milk package bags, disposable cups, plates, bowls	0.01- 7.8 mg/kg	Shen, 2005
Dolls or figures (7)	Not reported	BVL, 2008a
Selected toys and childcare products produced from foam plastic (8)	2.8 -1800 mg/kg	Danish EPA, 2006
Perfumes (20/36)	0.2 - 38 mg/kg	SCCP, 2007
Food		
Food packaged in cartons[1]	≤5 mg/kg	BfR, 2007
Popcorn (microwave)	29.4 mg/kg (max value)	BfR, 2007
Packaging material	7055.3 mg/kg (max value)	BVL, 2008
Bottled water (11 samples)	0.191 – 0.353 µg/l	Cao, 2007
Packed food (cheese, bread hazelnuts)	Not reported	Pfordt, 2004
Meals (8 samples)	0.02 – 0.07 mg/kg	Bopp and Altkofer, 2009

Other Media		
House dust	34 mg/kg (median)	Butte et al., 2008
Indoor air	390 ng/m³ (median)	Butte et al., 2008

[1] "fat-containing, powder and grain foods like rice, baking mixtures or breadcrumbs in paper and board packaging made of recycled fibers was particularly affected" (ECHA, 2009).

The overall consumer exposure information provided in ECHA (2009) is summarized as follows:

- Internal consumer exposure (estimated by Wormuth et al. (2006) using a scenario-based approach) accounts for 0.1 to 8 µg/kg BW per day for infants and 0.05 to 2 µg/kg BW per day in adults. Approximately 60% of exposure for infants and toddlers is covered by food, 30% by ingestion of soil/dust and 10% of indoor air. For adults, > 90% of the exposure to DiBP occurs via food intake and 10% through inhalation of indoor air.

- Fromme et al. (2007) estimated a daily uptake of DiBP ranging from 0.2 -14.9 µg/kg BW with a median value of 1.7 µg/kg BW and 95th percentile value of 5.27 µg/kg BW. The estimates were calculated using human urine excretion of MIBP using 399 single measurements in 50 adult volunteers on 6 consecutive days.

- Wittassek and Angerer (2007) calculated a mean daily intake of DiBP from excreted urine in persons 6 - 80 years old at 1.5 µg/kg BW and a maximum of 27.3 µg/kg BW.

- Huedorf (2007) calculated a daily intake of DiBP from urine excretion in a total of 111 children aged 6 years old, at 0.3 – 59.4 µg/kg BW, median of 2.2 µg/kg BW, and 95th percentile value of 10.99 µg/kg BW.

- In the Kinder-Unwelt-Survey (2008), the estimated daily intake in 600 children ages 3-14 years old was calculated using two different models. The calculations were based on volume-related and creatinine-related metabolite concentrations. The volume-based model intakes were: 3.8 µg/kg BW (median); 12.9 µg/kg BW (95th percentile); 70.8 µg/kg BW (maximum). The creatinine-based model intakes were: 3.0 µg/kg BW (median); 9.6 µg/kg BW (95th percentile); 33.4 µg/kg BW (maximum). The percentage

of children exceeding a daily intake of 10 µg/kg BW was 9.1% for volume-based model and 4.5% for the creatinine-based model.

- Fromme et al. (2007) calculated an intake assessment of dietary exposure for adults at 42 µg/day (median) and 157 µg/day (95th percentile) (based on duplicate diet samples).

- Wormuth et al. (2006) reported that dermal exposure as a result of contact with DiBP in cosmetics is considered to be negligible.

- The uptake in children calculated by urine excretion by Kinder-Unwelt-Survey (2008) and Huedorf (2007) exceed the mean internal uptakes in children 4 - 6 years old that was calculated by Wormuth et al. (2006) by factors of 5 to >7, indicating sources of unknown exposure.

7. DISCUSSION

Appendix A provides a summary of the NOAELs and LOAELs for organ-specific endpoints for oral exposure to DiBP. Studies for which effect levels for DiBP were derived include short-term (1-week) feeding and gavage studies in rats and mice (Zhu et al., 2010; Oishi and Hiraga, 1980a, c), 1- and 4-month feeding studies in rats (Hodge, 1954, 1953), and developmental toxicity studies in rats (Boberg et al., 2008; Howdeshell et al., 2008; Saillenfait et al., 2008, 2006, 2005; Borch et al., 2006; BASF, 2003, as cited by the European Commission, 2004).

The developing male reproductive tract is the most sensitive target of DiBP toxicity identified in the available studies, showing responses suggestive of an anti-androgen effect of DiBP in rats. The study by Saillenfait et al. (2008), in which litters were born and pups were observed into adulthood, provided the most sensitive evidence of anti-androgen effects of DiBP. Saillenfait et al. (2008) reported a significant decrease in male AGD, an increased incidence of thoracic areolas and/or nipples in male offspring, and a significant increase in the incidence of male offspring with testicular tubular degeneration characterized by atrophy and/or hypoplasia at a LOAEL of 250 mg/kg-day, with a corresponding NOAEL of 125 mg/kg-day. These effects increased in severity and incidence with increasing dose. Other anti-androgenic effects observed at higher dose levels in this study included decreased testes, seminal vesicle, and epididymis weights, and increased incidence of males with external reproductive tract malformations, including hypospadias, exposed os penis, nonscrotal testes, and azospermia.

Other developmental toxicity studies have also reported anti-androgenic effects in male rat offspring. Saillenfait et al. (2006) reported a significant disturbance in the degree of testicular migration observed in male rat fetuses from DiBP-exposed dams, and a significant increase in the incidence of male fetuses with ectopic (undescended) testes at ≥500 mg/kg-day. Boberg et al. (2008) reported a significant decrease in AGD as well as impaired testicular development among male rat fetuses from DiBP-exposed dams at 600 mg/kg-day. These effects on male rat reproductive tract development from gestational exposure to DiBP are similar to those observed in rats exposed to certain other phthalate esters (e.g., diethylhexyl phthalate and butylbenzyl phthalate), which are thought to be mediated via key events related to decreased production of testosterone leading to decreased levels of dihydrotestosterone and decreased expression of the *insl-3* gene (Foster, 2006). Significant decreases in fetal testosterone production following DiBP exposure in utero have been reported by Howdeshell et al. (2008) and Borch et al. (2006) at ≥300 mg/kg-day. Additionally, Boberg et al. (2008) reported decreased testicular levels of *insl-3* mRNA in DiBP-exposed male rat fetuses. DiBP impairment of the developing male reproductive tract is expected to be relevant to humans because rats and humans synthesize testosterone and dihydrotestosterone via the same enzymes (Foster, 2006). This expectation is weakly supported by available human studies that reported correlations between reduced AGD in male infants and maternal urinary levels of MIBP (Marsee et al., 2006; Swan et al., 2006, 2005) and altered male-associated play behavior in boys and maternal urinary levels of MIBP (Swan et al., 2010).

Other developmental effects reported in rats exposed to DiBP were generally observed at higher dose levels (>500 mg/kg-day) than the lowest doses associated with impaired male rat reproductive tract development; these effects include increased incidence of resorptions, reduced fetal growth, increased fetal mortality, and increased incidence of skeletal variations and external, visceral and skeletal malformations (Howdeshell et al., 2008; Borch et al., 2006; Saillenfait et al., 2006, 2005; BASF, 2003, as cited by the European Commission, 2004). Comparing available data across these studies suggests a temporal dependence on exposure for embryolethality and effects on fetal growth. Increased fetal mortality and decreased fetal growth were observed when rats were exposed to DiBP on GDs 6–21 (Howdeshell et al., 2008; Borch et al., 2006; Saillenfait et al., 2006, 2005; BASF, 2003, as cited by the European Commission, 2004), but not when rats were exposed to DiBP on GDs 12–21 (Saillenfait et al., 2008). These data suggest a correlation of these effects with early gestational exposure.

Maternal toxicity was characterized by decreased gestational weight gain in some of the developmental toxicity studies (Howdeshell et al., 2008; Saillenfait et al., 2006, 2005; BASF, 2003, as cited by the European Commission, 2004). These effects may be related to the corresponding reductions in fetal viability and growth also reported in these studies. The data summarized by the European Commission (2004) based on the unpublished BASF study (2003) suggested that impaired development of rat fetuses may have been confounded by effects on maternal food intake and body weight gain following dietary exposure to DiBP. However, gavage studies also reported effects on maternal weight gain, and effects on the developing male reproductive tract were also reported in the absence of effects on maternal weight gain by Saillenfait et al. (2008) and Borch et al. (2006).

No reproduction studies (one-, two-, or multigenerational studies) were identified for DiBP exposure by any route. However, limited data suggest that the adolescent and adult male reproductive tracts also appear to be targets of DiBP toxicity. Hodge (1954) reported a significant reduction in testes weights (↓ 45%, relative testes weights) of adult male albino rats exposed to 5,960 mg DiBP/kg-day in the diet for 4 months. The lowest LOAEL among those identified for adverse effects on the male reproductive tract of adolescent or adult rodents orally exposed to DiBP was 500 mg/kg-day for decreased testes weight and increased number of apoptotic spermatogenic cells in sexually immature Sprague-Dawley rats exposed to DiBP by gavage for 7 days (Zhu et al., 2010). Oishi and Hiraga (1980a) reported similar findings among adolescent male Wistar rats following short-term dietary exposure to 1,212 mg DiBP/kg-day. These rats exhibited significant reductions in testes weights accompanied by elevated testicular testosterone levels and marked inhibition of spermatogenesis and desquamation of spermatocytes. Similar short-term studies in mice reported different results. Zhu et al. (2010) reported a significant decrease in testes weight of adolescent mice following 7-day exposures to DiBP at doses ≥1,000 mg/kg-day, but no change in the numbers of apoptotic spermatogenic cells observed. Oishi and Hiraga (1980c) reported increased relative testes weight in mice at 2,083 mg/kg-day (possible reflecting decreased body weight in these animals) and no change in testicular testosterone levels. However, Oishi and Hiraga (1980a, c) reported significant decreases in testicular zinc concentrations among both rats and mice. Zinc is an essential element for maintenance of germinal epithelium in the testes, and zinc deficiency is associated with testicular atrophy (Oishi and Hiraga, 1980a).

Limited repeated-dose data are suggestive that the liver may represent another target of DiBP toxicity, but data are limited primarily to organ weight changes. Hodge (1954, 1953) observed significant elevations in absolute and relative liver weights in the absence of any

significant pathological changes in rats fed a diet containing ≥1,417 mg DiBP/kg-day for up to 4 months. Dietary exposure to DiBP for 1 week at doses of 1,212 or 2,083 mg/kg-day in adolescent rats and mice, respectively, resulted in elevated liver weights in both species (Oishi and Hiraga, 1980a, c). Histopathology was not performed in these studies.

Hodge (1953) also reported a significant increase in relative (but not absolute) kidney weight in the absence of any significant pathological changes in rats fed a diet containing 2,975 mg DiBP/kg-day for 1 month, but Hodge (1954) did not observe similar significant weight changes in the kidneys of rats exposed to up to 5,960 mg/kg-day for 4 months. No significant changes in kidney weights were reported in short-term studies of DiBP in rats (BUA, 1998, as cited by the European Commission, 2004, 2000; Oishi and Hiraga, 1980a), although a slight decrease in relative kidney weight was reported in a short-term study in mice (Oishi and Hiraga, 1980c).

Benchmark Dose (BMD) Analysis

The BMD method for generating acceptable daily intake levels (ADI's) is an alternative to methods that use NOAELs and LOAELs. A BMD is a dose at which a specified low incidence (i.e 10%) of health risk occurs over background levels (BMD_{10}). The $BMDL_{10}$ is the 95% lower confidence limit of the BMD_{10}. The BMD approach is thought to more accurately estimate a point of departure (POD) for each effect since it uses the entire dose-response curve and is independent of the doses tested.

To derive a $BMDL_{10}$, experimental data is curve fit with multiple statistical routines in order to estimate an effect dose level. The generated curves and associated statistics for each model routine are reviewed and the most appropriate endpoint chosen based on established criteria. The estimated dose level is then combined with uncertainty factors to generate an ADI.

For this report, toxicity endpoints for short- and intermediate-term incidental oral exposures to DiBP were selected from an extended developmental study by Saillenfait et al. (2008) and other developmental studies by Saillenfait et al. (2005, 2006) and Howdeshell et al. (2008). These data were used in a BMD approach for calculating ADI's. NOAELs and LOAELS from these studies (described above) were compared to the generated $BMDL_{10}$s.

BMD software designed by EPA (BMDS version 2.1.2) was used for BMD analysis of continuous data on DiBP induced changes in body weight (maternal, fetus, pup), organ weight

(testis, prostate, epididymides, seminiferous vesicles, etc), and reproduction (age of preputial separation, number pups born, fetal mortality, anogenital distance, testicular testosterone production, areolas/nipples er mature animal, and resorptions). BMD software was also used for analysis of dichotomous data on DiBP-induced changes in development (incidence of visceral, external, skeletal, and other malformations and variations) and reproduction (incidence of epididymal azoospermia, testicular interstitial cell hyperplasia, and testicular tubular necrosis, atrophy, degeneration, or hypoplasia). The data sets for these endpoints were thought to be of sufficient quality (dose-related, corroborated in multiple studies) to use in a BMD approach and were used to more accurately estimate a point of departure (POD) from each study for each effect.

BMD continuous models were selected to model data based on continuous variables (i.e. body weight). The $BMDL_{10}$ (95% lower confidence interval of the estimated benchmark dose that results in a 10% change) was estimated for continuous data using Linear, Polynomial, Hill, and Power models. For these endpoints, a 10% change was considered reasonable because most organ or body weight changes that are less than 10% are not associated with adverse effects. Results from each data set were screened to exclude model runs that had obviously misfitted curves, goodness-of fit p-values < 0.1, and a low $BMDL_{10}$ value to high $BMDL_{10}$ value ratio of > 3. Following this screening, model selection preference was given to runs with high p-values, low Akaike's Information Criterion (AIC), and data points near estimated BMD and BMDL levels.

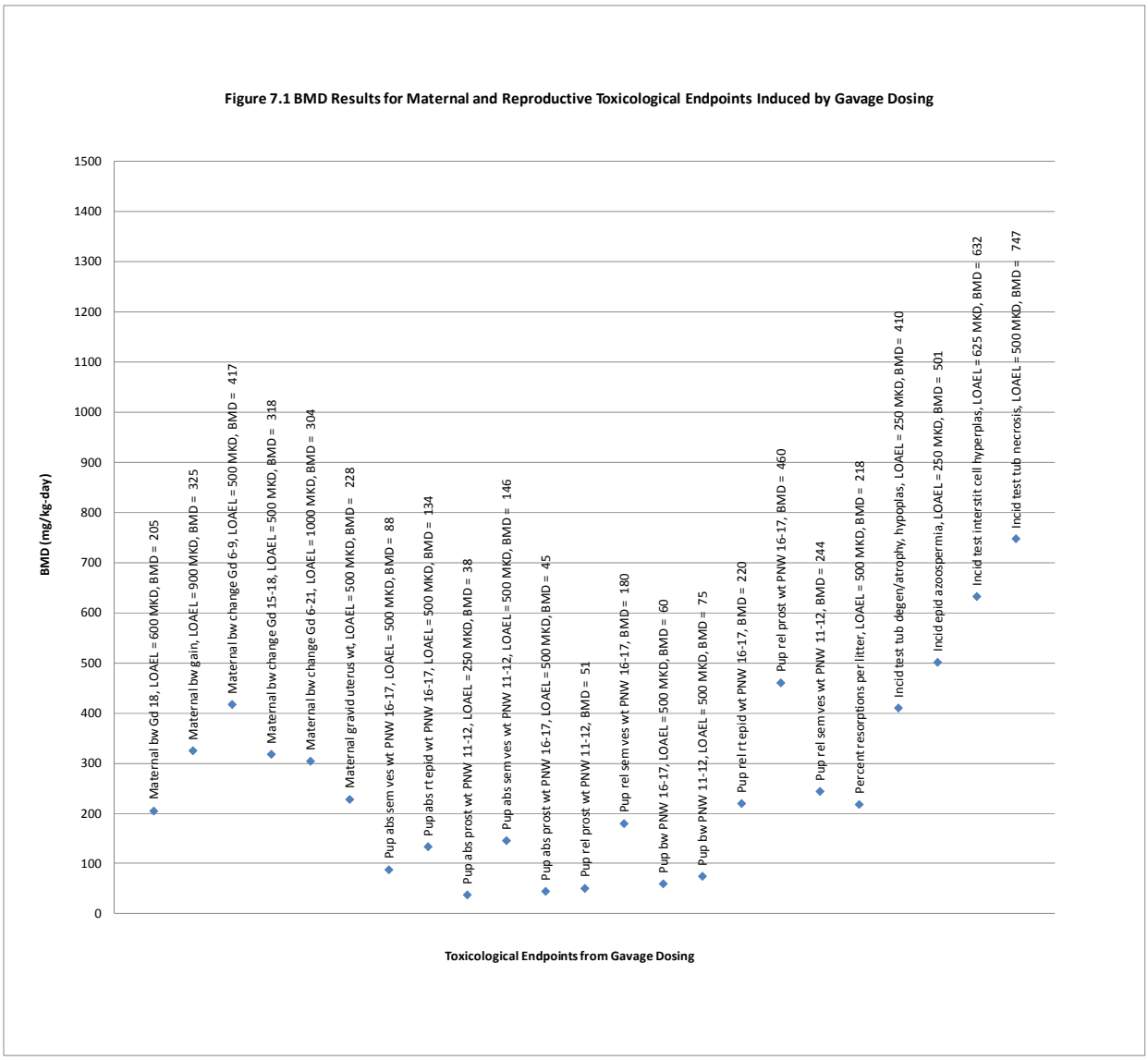
BMD dichotomous models (Gamma, Logistic, Multistage, Probit, and Weibull) were selected to graph data based on quantal variables. As with continuous data, the $BMDL_{10}$ was also estimated for these data. For these endpoints, a 10% change in a parameter was considered reasonable. The $BMDL_{10}$ is different than that used previously by a Chronic Hazard Advisory Panel convened by CPSC (2001; BMD_{05}), and CPSC staff (2002) for setting an ADIs based on quantal data (the incidence of spongiosis hepatis in rats) for diisononyl phthalate. Dichotomous results were screened as described above.

The BMD_{10} and $BMDL_{10}$ results of selected endpoints can be seen for gavage data (Figure 7.1, 7.2, 7.3, 7.4). Summarized BMD_{10} and $BMDL_{10}$ results and graphs can also be seen in Appendix C.

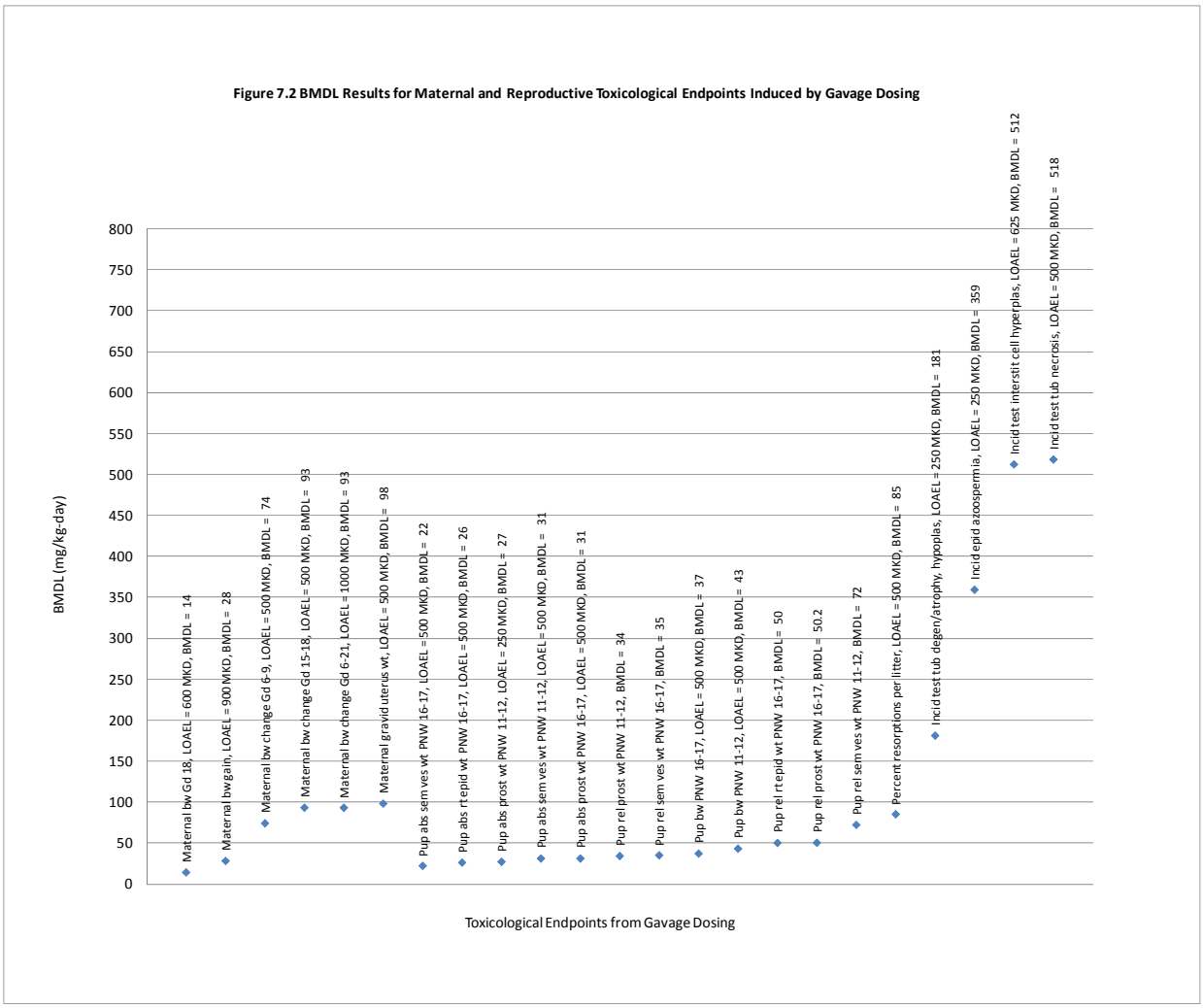
When looking at maternal and reproductive gavage dosing data, $BMDL_{10}$s ranged from 14 – 93 mg/kg-day for decreases in maternal body weight and gravid uterus weight, 22 – 72

mg/kg-day for absolute and relative changes in reproductive organ weight, and 181 – 518 mg/kg-day for increased incidence of various testicular pathologies (Figure 7.2).

When looking at developmental gavage dosing data, $BMDL_{10}$s range from 9.8 – 43 mg/kg-day for developmental issues involving the reproductive system, 35 – 70 mg/kg-day for changes in fetus or pup body weights, and 220 – 968 mg/kg-day for developmental pathologies involving the visceral or skeletal systems (Figure 7.4).

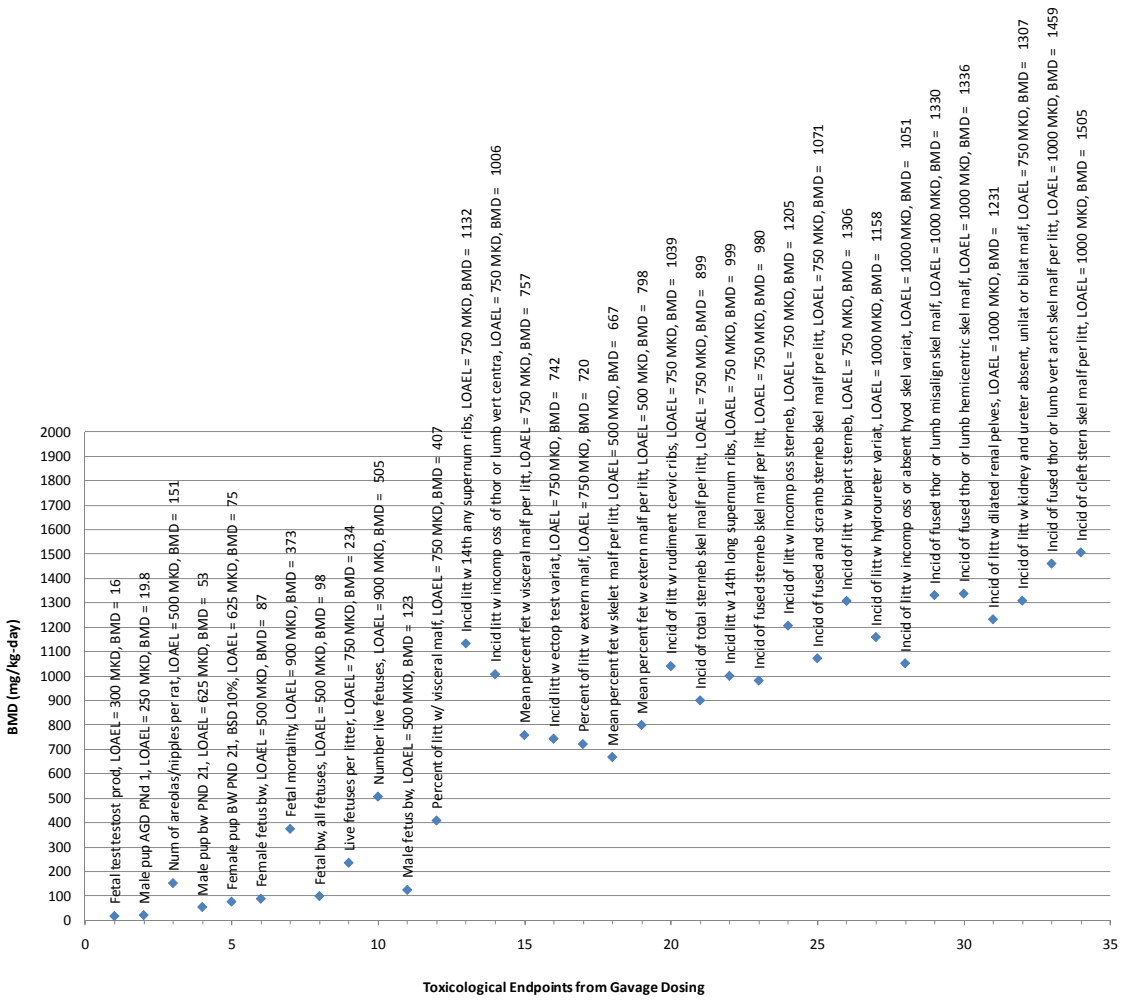


MKD = mg/kg-day; BSD = Biologically significant difference

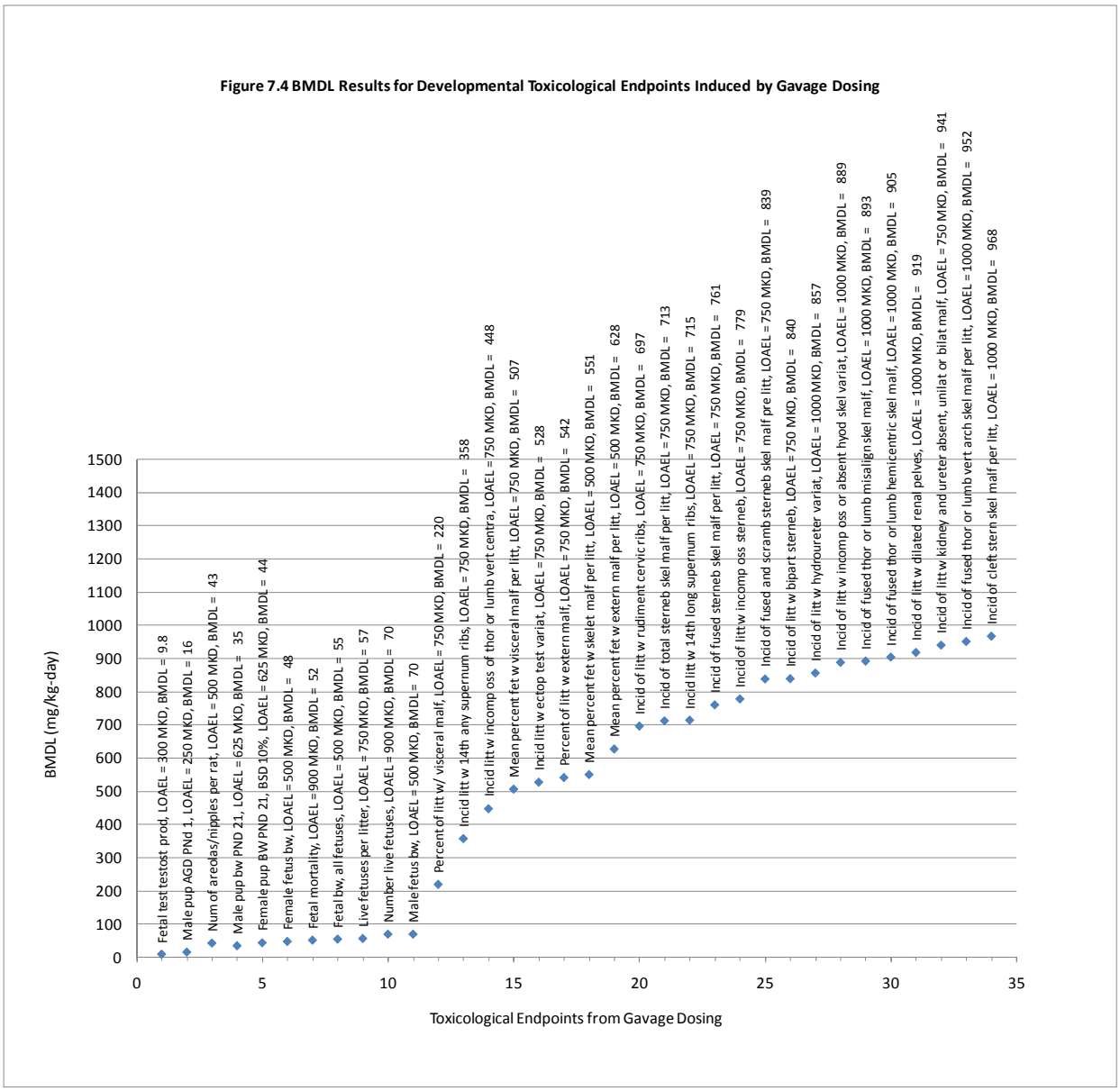


MKD = mg/kg-day; BSD = Biologically significant difference

Figure 7.3 BMD Results for Developmental Toxicological Endpoints Induced by Gavage Dosing



MKD = mg/kg-day; BSD = Biologically significant difference



MKD = mg/kg-day; BSD = Biologically significant difference

Overall Acceptable Daily Intakes

Acceptable daily intakes values (ADI's) are calculated when a given chemical is considered "toxic" and sufficient toxicity information is available. The ADI is the amount of a chemical that one may be exposed to on a daily basis without posing a significant risk of health effects to consumers. ADI's were estimated for short-term exposure durations for the general population (non-reproductive endpoint) and short-term exposures to immature animals (reproductive endpoint). An additional short-term ADI was estimated for developmental effects (maternal exposures resulting in developmental effects).

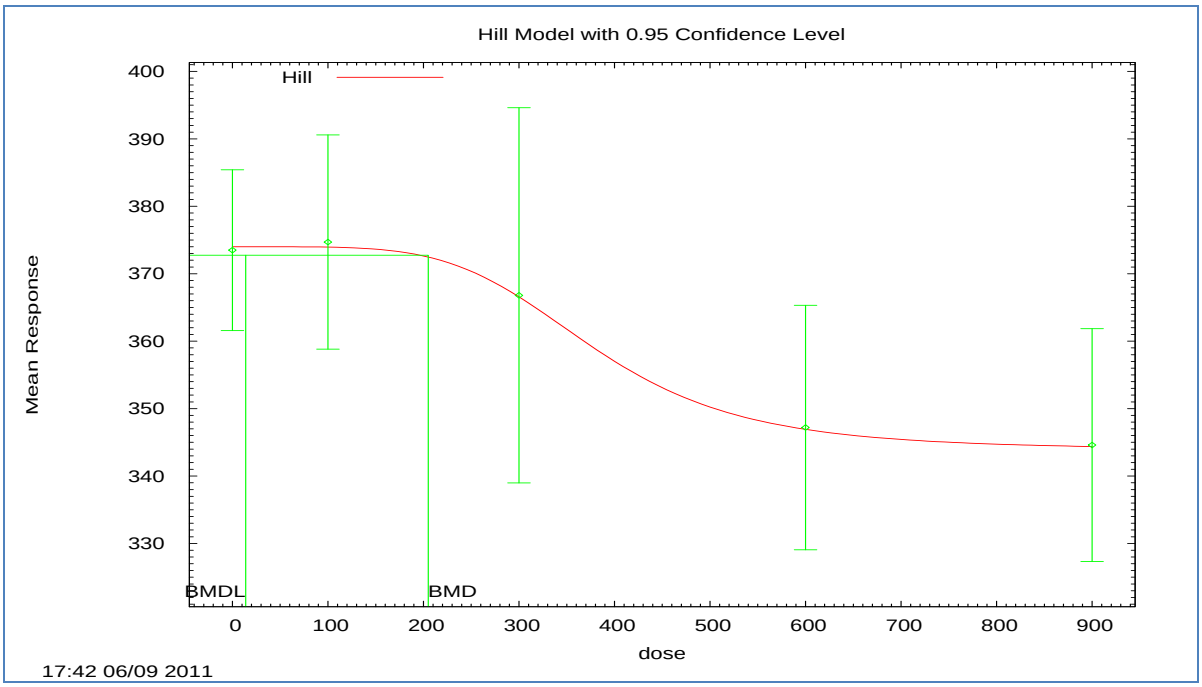
General population ADI's

Short-term oral exposures – general population

For short-duration oral exposures, the $BMDL_{10}$ of 14 mg/kg-day (Howdeshell et al., 2009) was chosen as the representative overall hazard endpoint for general toxicity. This endpoint was derived from a gestational exposure study in which pregnant female Sprague-Dawley rats were gavage dosed with DiBP during gestation days 8 to 18.

DiBP doses of 600 mg/kg-day (LOAEL; NOAEL = 300 mg/kg-day) significantly decreased the body weight of Sprague-Dawley dams on gestation day 18. $BMDL_{10}$ model calculations suggested that the decrease in body weight was best described by the Hill model (AIC = 190.7, model dependency ratio = 2.0 [< 3], goodness of fit p-value = 0.89; see Figure 7.5 below).

Figure 7.5 Hill Model Plot of Dam Body Weight on Gd 18 (Howdeshell et al., 2008)



Choice of body weight study data for use as a hazard endpoint induced by short-term DiBP exposure was supported by additional body weight data that had slightly higher hazard effect levels. Calculated $BMDL_{10}$s for other changes in maternal body weight ranged from 28 to 93 (LOAELs = 500 to 1000 mg/kg-day).

The $BMDL_{10}$ of 14 mg/kg-day was used to generate an Acceptable Daily Intake (ADI) by dividing by an uncertainty factor of 100 (10X for interspecies variation, 10X for intraspecies variation and sensitive populations). This "safety factor" is typically applied by CPSC to the lowest NO[A]EL for animal data in which developmental, reproductive, or neurotoxicological effects have been determined (16 CFR§1500.135(d)(4)(B)). **The short-term exposure oral ADI for the general population was calculated to be 0.14 mg/kg-day.**

Reproductive ADI

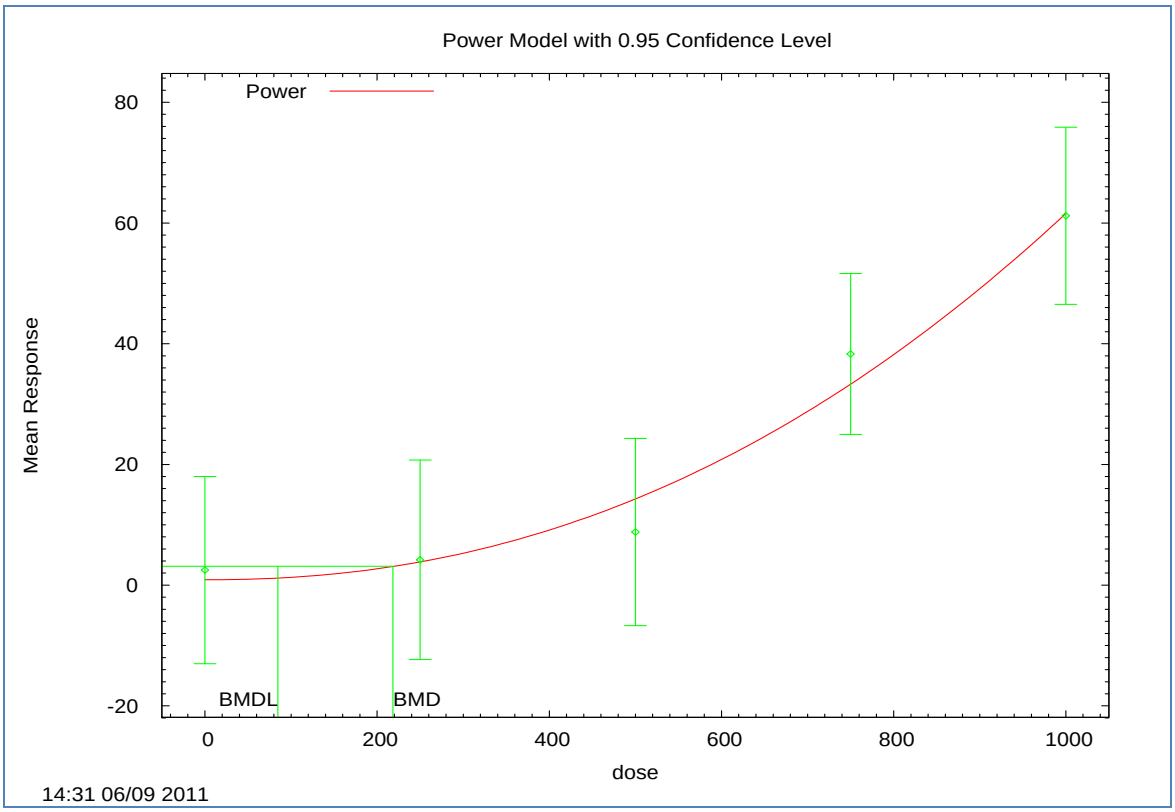
Short-term oral exposures – reproduction

For short-duration oral exposures and reproductive endpoints, the $BMDL_{10}$ of 85 mg/kg-day (Saillenfait et al., 2005; BMD = 218 mg/kg-day) was chosen as the representative hazard

endpoint. This endpoint was derived from a developmental toxicity study in which female Sprague-Dawley rats were gavage dosed with DiBP during gestation day 6 to 20.

DiBP doses of 500 mg/kg-day (LOAEL; NOAEL = 250 mg/kg-day) significantly increased the percent resorptions per litter. $BMDL_{10}$ model calculations suggested that the increase in resorptions was best described by the Power model (AIC = 426.4, model dependency ratio = 2.8 [< 3], goodness of fit p-value = 0.50; see Figure 7.6 below).

Figure 7.6 Power Model Plot of Percent Resorptions per Litter (Saillenfait et al., 2005)



Choice of percent resorption data was supported by additional reproduction-related data with a slightly higher hazard effect level. Decreased gravid uterus weight ocurred at a LOAEL of 500 mg/kg-day ($BMDL_{10}$ = 98 mg/kg-day; NOAEL = 250 mg/kg-day).

The $BMDL_{10}$ of 85 mg/kg-day was used to generate an Acceptable Daily Intake (ADI) by dividing by an uncertainty factor of 100 (10X for interspecies variation, 10X for intraspecies variation and sensitive populations). This “safety factor” is typically applied by CPSC to the lowest NO[A]EL for animal data in which developmental, reproductive, or neurotoxicological

effects have been determined (16 CFR§1500.135(d)(4)(B)). **The long-term exposure oral ADI for the general population was calculated to be 0.85 mg/kg-day.**

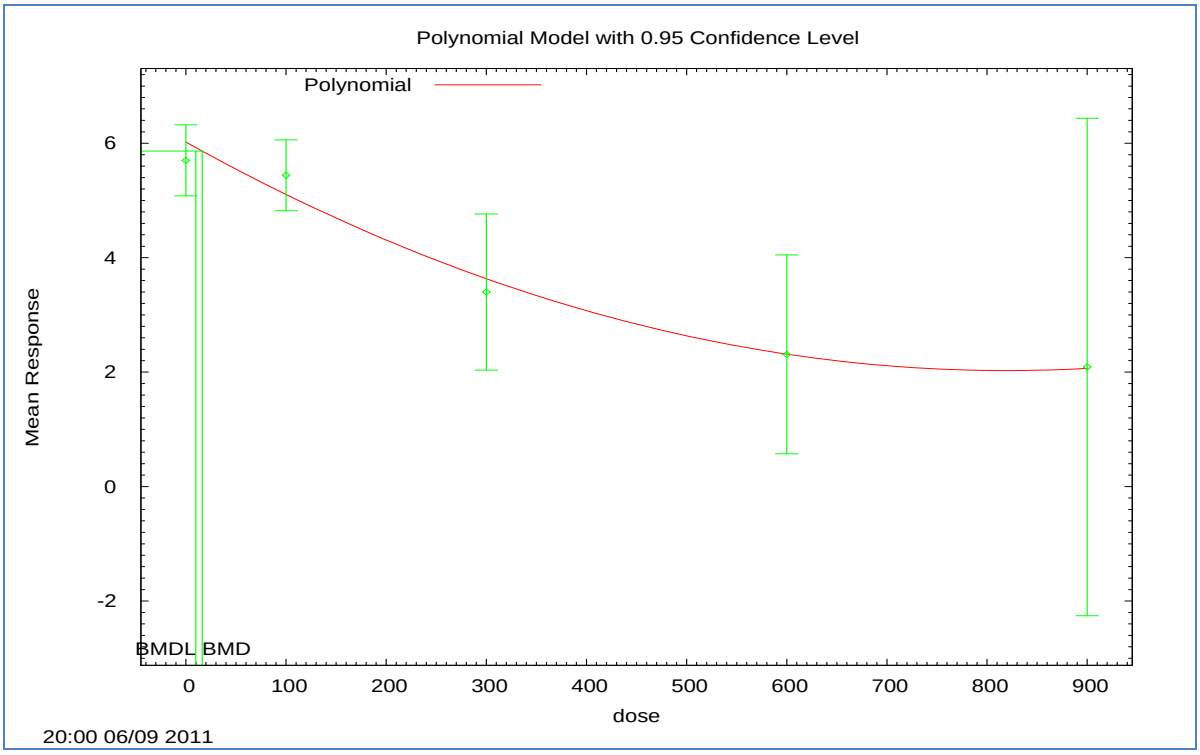
Developmental ADI

Maternal exposures – developmental effects

For developmental effects, the maternal dose $BMDL_{10}$ of 9.8 mg/kg-day was chosen as the representative overall hazard endpoint (Howdeshell et al., 2008)**.** This endpoint was derived from a gestational exposure study in which pregnant female Sprague-Dawley rats were gavage dosed with DiBP during gestation days 8 to 18.

DiBP doses of 300 mg/kg-day (LOAEL; NOAEL = 100 mg/kg-day) significantly decreased the production of testicular testosterone in Gd 18 male fetuses. $BMDL_{10}$ model calculations suggested that the decrease in body weight was best described by the Power model (AIC = 60.9, model dependency ratio = 2.7 [< 3], goodness of fit p-value = 0.71; see Figure 7.7 below).

Figure 7.7 Polynomial Model Plot of Fetal Testicular Testosterone Production (Howdeshell et al., 2008)



Choice of fetal testicular testosterone production data was supported by additional developmental-related data with a higher hazard effect levels. The $BMDL_{10}$s calculated for changes in anogenital distance and the number of areolas/nipples per rat were 16 and 43 mg/kg-day, respectively (LOAEL = 250 – 500 mg/kg-day). Decreases in pup absolute and relative seminal vesicle, epididymis, prostate, and body weights (at PNW 11 – 12 or 16 – 17) ranged from 22 to 72 mg/kg-day (LOAEL = 250 – 500 mg/kg-day). The range of $BMDL_{10}$s (37 – 70 mg/kg-day; LOAEL = 500 – 900 mg/kg-day) calculated for decrements in fetal or young pup weights (PND21), the number of live fetuses per litter, and increases in fetal mortality were similar to those presented for older pups. Pathological changes in reproductive tissues (increased incidence of testicular tubule degeneration/atrophy, hypoplasia, epididymal azoospermia, testicular interstitial cell hyperplasia, and testicular tubule necrosis) had slightly higher $BMDL_{10}$ effect levels than these endpoints (181 – 518 mg/kg-day; LOAEL = 250 – 625 mg/kg-day). Classical developmental effects occurred at an even dose higher $BMDL_{10}$ range (220 – 968 mg/kg-day; LOAEL = 500 – 1000 mg/kg-day).

The $BMDL_{10}$ of 9.8 mg/kg-day was used to generate an Acceptable Daily Intake (ADI) by dividing by an uncertainty factor of 100 (10X for interspecies variation, 10X for intraspecies

variation and sensitive populations). This “safety factor” is typically applied by CPSC to the lowest NO[A]EL for animal data in which developmental, reproductive, or neurotoxicological effects have been determined (16 CFR§1500.135(d)(4)(B)). **The developmental ADI was calculated to be 0.098 mg/kg-day.**

Other ADIs

Insufficient evidence (hazard data) precluded the generation of ADI’s for inhalation or dermal exposures or for cancer endpoints.

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Appendix A. Summary of Endpoints by Organ System

Table A.1. Summary of NOAELs/LOAELs Identified for DiBP by Organ System

Species (Gender)	Exposure Route	Dose (mg/kg-day) (Number of Animals per Dose Group)	Dose Duration	Effect Category	Toxicological Endpoint (mg/kg-day)	Toxicological Basis	Citation
Wistar rats (5 weeks old) (M)	Oral, diet	0 or 1,212 (10 treated rats, 20 control rats)	1 week	General	NOAEL=1,212 LOAEL=None	Slight nonsignificant ↓ in body weight (6% lower than controls) and food consumption	Oishi and Hiraga, 1980a
				Liver	NOAEL=None LOAEL=1,212	↑ liver weight, ↓ liver zinc levels	
				Kidney	NOAEL=1,212 LOAEL=None	No significant changes in kidney weight or kidney zinc levels	
				Testes	NOAEL=None LOAEL=1,212	↓ absolute and relative testes weight, ↓ testicular zinc levels, ↑ testicular testosterone levels, ↓ spermatogenesis, desquamation of spermatocytes	
JCL:ICR mice (5 weeks old) (M)	Oral, diet	0 or 2,083 (10 M mice per group)	1 week	General	NOAEL=None LOAEL=2,083	↓ body weight	Oishi and Hiraga, 1980c
				Liver	NOAEL=None LOAEL=2,083	↑ liver weight, ↓ liver zinc levels	
				Kidney	NOAEL=None LOAEL=2,083	↓ kidney weight (no significant change in kidney zinc levels)	
				Testes	NOAEL=None LOAEL=2,083	↑ relative testes weight and ↓ testicular zinc levels; no effects on testicular testosterone levels (no histological examination of reproductive tract)	
Sprague-Dawley rats (21 days old) (M)	Oral, gavage	0, 100, 300, 500, 800, or 1,000 (group size not specified)	1 week	Testes	NOAEL=300 LOAEL=500	↓ testes weight, ↑ number of apoptotic (TUNEL-positive) spermatogenic cells per seminiferous tubule	Zhu et al., 2010

Table A.1. Summary of NOAELs/LOAELs Identified for DiBP by Organ System							
Species (Gender)	**Exposure Route**	**Dose (mg/kg-day) (Number of Animals per Dose Group)**	**Dose Duration**	**Effect Category**	**Toxicological Endpoint (mg/kg-day)**	**Toxicological Basis**	**Citation**
C57BL/6N mice (21 days old) (M)	Oral, gavage	0, 100, 300, 500, 800, or 1,000 (group size not specified)	1 week	Testes	NOAEL=800 LOAEL=1,000	↓ testes weight; no changes in the number of apoptotic (TUNEL-positive) spermatogenic cells per seminiferous tubule	Zhu et al., 2010
Albino rats (M)	Oral, diet	0, 15, 142, 1,417, 2,975, or 8,911 (5 M rats per group)	1 month	General	NOAEL=2,975 LOAEL=8,911	↓ body weight	Hodge, 1953
				Liver	NOAEL=142 LOAEL=1,417	↑ liver weight (no corresponding histological changes)	
				Kidney	NOAEL=1,417 LOAEL=2,975	↑ relative but not absolute kidney weight (no corresponding histological changes)	
Albino rats (M&F)	Oral, diet	0, 67, 738, or 5,960 (5 M rats per group); 0, 85, 795, or 4,861 (5 F rats per group)	4 months	General	NOAEL=738–795 LOAEL=4,861–5,960	↓ body weight	Hodge, 1954
				Blood	NOAEL=4,861–5,960 LOAEL=None	No significant hematological changes	
				Liver	NOAEL=738–795 LOAEL=4,861–5,960	↑ liver weight (no corresponding histological changes)	
				Kidney	NOAEL=4,861–5,960 LOAEL=None	No significant changes in kidney weight or kidney pathology	
				Testes	NOAEL=738 LOAEL=5,960	↓ absolute and relative testes weights (histopathology was not performed)	
Wistar rats (F)	Oral gavage	0, 88, 363, or 942 (25 F rats per group)	GDs 6–20; examined on GD 20	General	NOAEL=363 LOAEL=942	↓ food intake and body weight gain in dams	BASF, 2003, as cited by the European Commission, 2004
				Development/ fetus	NOAEL=363 LOAEL=942	↓ fetal weight, ↑ skeletal variations	

Table A.1. Summary of NOAELs/LOAELs Identified for DiBP by Organ System							
Species (Gender)	**Exposure Route**	**Dose (mg/kg-day) (Number of Animals per Dose Group)**	**Dose Duration**	**Effect Category**	**Toxicological Endpoint (mg/kg-day)**	**Toxicological Basis**	**Citation**
Sprague-Dawley rats (F)	Oral gavage	0, 100, 300, 600, or 900 (5–8 F rats per group)	GDs 8–18; examined on GD 18	General	NOAEL=600 LOAEL=900	↓ body weight gain in dams	Howdeshell et al., 2008
				Development/ fetus	NOAEL=100 LOAEL=300	↓ fetal testicular testosterone production; also ↑ resorptions and ↑ fetal mortality at higher doses	
Sprague-Dawley rats (F)	Oral gavage	0, 250, 500, 750, or 1,000 (10–14 F rats per group)	GDs 6–20; examined on GD 20	General	NOAEL=500 LOAEL=750	↓ body weight gain in dams	Saillenfait et al., 2005
				Development/ fetus	NOAEL=250 LOAEL=500	↑ resorptions; also ↑ fetal mortality, ↓ fetal body weight, and ↑ incidence of litters with fetuses with undescended testes at higher doses	
Sprague-Dawley rats (F)	Oral gavage	0, 250, 500, 750, or 1,000 (20–22 F rats per group)	GDs 6–20; examined on GD 20	General	NOAEL=250 LOAEL=500	↓ body weight gain and ↑ gravid uterine weight in dams	Saillenfait et al., 2006
				Development/ fetus	NOAEL=250 LOAEL=500	↓ fetal body weight and ↑ transabdominal testicular migration; also ↑ resorptions, ↑ incidence of undescended testes, and ↑ visceral or skeletal malformations/variations at higher doses	
Sprague-Dawley rats (F)	Oral gavage	0, 125, 250, 500, or 625 (11–13 F rats per group)	GDs 12–21; examined on PNDs 1, 4, 7, 14, and 21, and at PNWs 11–12 and 16–17	General	NOAEL=625 LOAEL=None	No maternal effects	Saillenfait et al., 2008
				Development/ fetus	NOAEL=125 LOAEL=250	↓ AGD (PND 1), retained areolas/nipples (PNDs 12–14 and PNWs 11–12/16–17), and ↑ testicular degeneration (PNWs 16–17) in male offspring; also delayed PPS; ↓ testes and epididymis weights and ↑ incidence of external reproductive tract malformations (hypospadias, nonscrotal testes, exposed os penis and azospermia) in male offspring at higher doses	

Table A.1. Summary of NOAELs/LOAELs Identified for DiBP by Organ System							
Species (Gender)	**Exposure Route**	**Dose (mg/kg-day) (Number of Animals per Dose Group)**	**Dose Duration**	**Effect Category**	**Toxicological Endpoint (mg/kg-day)**	**Toxicological Basis**	**Citation**
Wistar rats (F)	Oral gavage	0 or 600 (8 F rats per group)	GDs 7–21; examined on GDs 19 or 20/21	General	NOAEL=600 LOAEL=None	No maternal effects	Boberg et al., 2008; Borch et al., 2006
				Development/ fetus	NOAEL=None LOAEL=600	↓ fetal body weight, ↓ male AGD, ↓ testicular testosterone levels, and ↑ testicular lesions (clustering of small Leydig cells and Sertoli cell vacuolization)	

F = female; M = male; TUNEL = transferase dUTP nick end labeling

Appendix B. Critical Study Reviews

Oishi and Hiraga (1980a, b, c, d)

Oishi and Hiraga (1980a, b, c, d) evaluated the effects of 1-week dietary exposures to 2% DiBP (>98% pure) or the monoester MIBP (>98% pure) in 5-week-old male Wistar rats and JCL:ICR mice (10/species/group). For DiBP, using average body weights over the week-long studies of 132 and 24 g for rats and mice, respectively, and assuming default food consumption rates of 0.008 kg/day for male Wistar rats and 0.0025 kg/day for male $B6C3F_1$ mice (U.S. EPA, 1988), the corresponding doses were estimated as 1,212 and 2,083 mg/kg-day for rats and mice, respectively. For MIBP, average body weights were 145 and 25 g, respectively, for rats and mice, and doses were estimated as 1,103 and 2,083 mg/kg-day. Control groups of 10–20 animals were tested simultaneously with each material. Body, liver, kidney, and testes weights were measured in both species following the 1-week exposures. Zinc concentrations were measured in liver, kidney, testes, and serum, as were testosterone concentrations in the testes and serum. Histopathological examination was performed for rat testes only.

Treatment with DiBP resulted in slight interference with food consumption, a slight but nonsignificant reduction in rat body weight (6% lower than controls), a significant reduction in mouse body weight (13% lower than controls), significant increases in liver weights in both rats (27 and 35% higher than controls based on absolute and relative weights, respectively) and mice (45% higher than controls based on relative weight), a significant decrease in kidney weight in mice (10% lower than controls) with no change in kidney weight in rats, significant decreases in absolute and relative testes weight in rats (37% and 33%, respectively), and increased relative testes weight (29%) in mice (Tables B.1 and B.2). Liver zinc levels in the testes and liver were significantly decreased in both rats and mice. There were no significant changes in kidney or serum zinc levels in either species. Testosterone levels in the testes were increased in rats, but not mice. Testosterone levels in the serum were not elevated. Gross examination of the rat testes showed a large decrease in size relative to controls. Microscopy of rat testes revealed marked inhibition of spermatogenesis and desquamation of spermatocytes. The tested DiBP dose level of 1,212 mg/kg-day in rats is a LOAEL for increased liver weight and severe testicular atrophy in this study. The tested dose level of 2,083 mg/kg-day in mice is a LOAEL for decreased body weight and increased liver weight.

Table B.1. Body and Organ Weights and Zinc and Testosterone Levels in Male JCL:Wistar Rats Exposed to DiBP in the Diet for 1 Week

Endpoint	DiBP Dose (mg/kg-day)		MIBP Dose (mg/kg-day)	
	0	1,212	0	1,103
Final body weight (g)	165.7 ± 10.5[a]	155.6 ± 8.86	183 ± 7.1	165 ± 11.0[b]
Absolute organ weights (g)				
Testes	1.45 ± 0.31	0.91 ± 0.33[b]	1.73 ± 0.20	0.91 ± 0.16[b]
Liver	7.88 ± 0.58	9.99 ± 0.78[b]	NR	NR
Kidney	1.71 ± 0.14	1.63 ± 0.14	NR	NR
Relative organ weights[c]				
Testes	0.87 ± 0.16	0.58 ± 0.19[b]	0.94 ± 0.09	0.56 ± 0.08[b]
Liver	4.76 ± 0.26	6.42 ± 0.33[b]	NR	NR
Kidney	1.03 ± 0.05	1.05 ± 0.05	NR	NR
Zinc concentration[d]				
Testes	19.9 ± 2.48	17.7 ± 2.88[b]	–	72%[b]
Liver	29.0 ± 5.02	24.1 ± 2.54[b]	–	90%[b]
Kidney	19.7 ± 1.91	18.2 ± 1.49	–	102%
Serum	1.21 ± 0.91	1.14 ± 0.14	–	98%
Testicular testosterone[e]	–	250%[b]	–	260%[b]

[a]Mean ± SD for 10 rats (high dose DiBP and both doses MIBP) or 20 rats (control DiBP).
[b]Significantly different from controls, $p < 0.05$.
[c]Values are expressed as g per 100 g of body weight.
[d]Values for DiBP are expressed as μg/g of wet tissue or μg/mL of serum; values for MIBP are expressed as percentage of control and estimated from data presented graphically.
[e]Values are expressed as the percentages of control and estimated from data presented graphically.

NR = not reported

Sources: Oishi and Hiraga (1980a, b).

Table B.2. Body and Organ Weights and Zinc and Testosterone Levels in Male JCL:ICR Mice Exposed to DiBP in the Diet for 1 Week

Endpoint[a]	DiBP Dose (mg/kg-day)		MIBP Dose (mg/kg-day)	
	0	2,083	0	2,083
Final body weight (g)	29.4 ± 0.38	25.7 ± 0.47[b]	30.0 ± 0.24	26.2 ± 0.55[b]
Relative organ weights[c]				
Testes	0.633 ± 0.021	0.815 ± 0.039[b]	0.65 ± 0.03	0.94 ± 0.10[d]
Liver (with gall)	7.23 ± 0.09	10.5 ± 0.10[b]	7.33 ± 0.12	9.51 ± 0.21[d]
Kidneys	1.84 ± 0.06	1.66 ± 0.03[b]	1.86 ± 0.05	1.76 ± 0.03
Zinc concentration[d]				
Testes	–	76 ± 1.49[b]	–	64 ± 2.5[b]
Liver	–	91 ± 2.89[b]	–	101 ± 1.7
Kidney	–	104 ± 7.84	–	109 ± 1.1
Testicular testosterone[d]	–	107 ± 26	–	17 ± 3.8[e]

[a]Values are means ± SE for 10 animals.
[b]Significantly different from controls, $p < 0.05$ (Student's t-test).
[c]Values are expressed as the percentages of body weight.
[d]Values are expressed as the percentages of control.
[e]Significantly different from controls, $p < 0.05$ (Scheffe's method).

Sources: Oishi and Hiraga (1980c, d).

Results with MIBP were similar to those with DiBP in rats for the endpoints that were reported (liver and kidney weights were measured, but not reported) (see Table B.1). MIBP also produced results similar to DiBP in mice, except that testicular testosterone was significantly decreased to 17% of control levels in the mice treated with MIBP (Table B.2).

Foster et al. (1981)

Foster et al. (1981) evaluated the effects of MIBP exposure on the male reproductive tract in rats. In this study, MIBP (purity not reported) was administered as the ammonium salt (pH 6.0) in an aqueous solution by gavage at 0 or 800 mg/kg-day to groups of male Sprague-Dawley rats (6/group, age not reported) for 6 consecutive days. Rats were sacrificed on the day following the last dose. Testes and accessory sex organs were weighed and examined microscopically. Absolute and relative testes weights were significantly lower among MIBP-treated rats (approximately 40% decreased compared with controls, $p < 0.001$). Changes in seminal vesicle and prostate weights were not significantly different from controls. Histological examination of the testes revealed marked atrophy of the majority of the seminiferous tubules with a diminution of both spermatocytes and spermatogonia. Lesions

appeared bilateral in origin. No abnormalities were detected in sections of prostate or seminal vesicles. Additionally, urinary excretion of radiolabeled zinc was increased in MIBP-treated rats relative to controls and testicular zinc levels were significantly decreased compared to controls. Foster et al. (1981) tested several phthalates and observed that only those that affected zinc metabolism induced testicular effects.

Zhu et al. (2010)

Zhu et al. (2010) administered DiBP (99.9% pure) in corn oil to groups of 21-day-old male Sprague-Dawley rats or C57B1/6N mice via gavage at 0, 100, 300, 500, 800, or 1,000 mg/kg-day either once or daily for 7 consecutive days. Animals were sacrificed 1 day following the end of exposure and their testes were weighed and evaluated for apoptosis of spermatogenic cells using the in situ TUNEL method. An additional group of rats administered DiBP at 500 mg/kg-day for 7 days was sacrificed for vimentin immunohistochemistry analysis. A recovery experiment was also conducted whereby Sprague-Dawley rats administered DiBP once via gavage at 1,000 mg/kg were sacrificed at 1 (D1) to 8 (D8) days following exposure.

Data on testes weights and percentage of apoptotic spermatogenic cells were provided graphically by Zhu et al. (2010). Testes weights were comparable at all doses between treated and control animals (both rats and mice) administered DiBP only once. However, significant increases in the numbers of apoptotic spermatogenic cells per seminiferous tubule were observed in rats at ≥500 mg/kg ($p < 0.001$) and in mice at 800 mg/kg ($p=0.071$) in the single dose study. Rats treated for 7 consecutive days exhibited both a significant reduction ($p < 0.001$) in testes weight and a significant increase ($p < 0.05$) in the numbers of apoptotic spermatogenic cells at ≥500 mg/kg compared to controls. Additionally, immunohistochemistry of rats exposed to 500 mg/kg-day revealed disorganized or reduced vimentin filaments in the perinuclear and basal regions of Sertoli cells and sloughing of apoptotic spermatocytes from the epithelium. In the recovery experiment, rats exposed to 1,000 mg DiBP/kg once exhibited significant reductions in testes weights at D2 ($p < 0.05$) and D5 ($p < 0.001$) compared to controls. However, testes weights of exposed rats recovered to within control levels by D6. A similar pattern of recovery was observed based on increases in apoptotic spermatogenic cells, whereby a significant increase in comparison to controls observed on D1, D2, and D5 recovered to control levels by D6. Mice treated for 7 consecutive days only exhibited a significant decrease ($p < 0.01$) in testes weight at 1,000 mg/kg-day. These results indicate acute NOAEL and LOAEL values of 300 and 500 mg/kg-day, respectively, for rats based on decreased testes weights, increased apoptosis of spermatogenic cells, and changes in the distribution of vimentin filaments. For mice, acute

NOAEL and LOAEL values of 800 and 1,000 mg/kg-day, respectively, are identified for decreased testes weights.

BUA (1998)

In a German study submitted by BUA (1998) to the European Commission (2004, 2000), groups of female F344 rats (5/group) were administered DiBP (in corn oil, purity not reported) via gavage doses of 0, 50, 100, 200, or 2,000 mg/kg-day for 14 days. Rats were monitored for changes in food intake and body weight, clinical signs of toxicity, hematology (parameters not specified), clinical chemistry (albumin, triglyceride, cholesterol levels), organ weights (liver and kidney), and gross pathology. Additionally, livers were collected and measured for protein and cytochrome P-450 content, as well as ethoxyresorufin O-dealkylase, pentoxyresorufin O-dealkylase, p-nitrophenol hydroxylase, and dodecanoic acid 12-hydroxylase activities. Data were only reported qualitatively in the available secondary sources. No significant effects on body weights or food consumption were reported, and no significant clinical signs were noted. At the highest dose, DiBP-treated rats experienced elevated absolute and relative liver weights, increased serum albumin levels, and decreased triglyceride and cholesterol levels. Serum triglyceride levels were also decreased in the 100 and 200 mg/kg-day groups. Signs of peroxisome proliferation in hepatic tissue were evident at ≥100 mg/kg-day (based on an "increase of biochemical parameters"). No other quantitative information was provided in the available secondary sources. The European Commission (2004, 2000) summary documents identified a NOAEL of 50 mg/kg-day, but the reporting for this study is inadequate to identify reliable acute NOAEL or LOAEL values for hazard identification purposes.

Hodge (1954, 1953)

Hodge (1954, 1953) evaluated the effects of dietary DiBP (purity not reported) on groups of weanling albino rats exposed for 1 or 4 months. In the 1-month study, groups of male albino rats (5/group, weanling) were maintained on diets containing 0, 0.01, 0.1, 1.0, 2.0, or 5.0% DiBP (Hodge, 1953). Using the average body weights of the rats reported in the study (139, 124, 127, 127, 121, and 101 g at 0, 0.01, 0.1, 1.0, 2.0, and 5.0%, respectively) and a reference value for food consumption (0.018 kg/day) for male rats of an unspecified strain (U.S. EPA, 1988), these concentrations correspond to doses of 0, 15, 142, 1,417, 2,975, or 8,911 mg/kg-day. Mortality and weekly body weight gain were monitored. At termination, gross necropsy was performed, and liver and kidneys from all animals were weighed and examined histologically.

Other than one control rat that became sick and died during the study, all rats survived to study termination (Hodge, 1953). Table B.3 summarizes the significant changes based on terminal body weights and liver and kidney weights observed in rats maintained on a diet containing DiBP for 1 month. Growth curves for the groups exposed to ≤1,417 mg/kg-day were similar to each other; terminal body weights of these rats were approximately 14–16% lower than controls. However, the author reported that several control rats experienced exceptionally rapid growth, which compromised comparisons with the treatment groups. Compared to the groups exposed to ≤1,417 mg/kg-day, the growth curve of the group exposed to 2,975 mg/kg-day was consistently lower throughout the study, and the average terminal body weight was approximately 5% lower. Rats exposed to 8,911 mg/kg-day in the diet clearly demonstrated reduced growth, and terminal body weight was approximately 62% of controls and 75% of the other dose groups. Absolute and relative liver weights were elevated in rats exposed to ≥1,417 mg/kg-day in the diet. Absolute kidney weights were unchanged, but relative kidney weights were significantly elevated over controls in rats exposed to ≥2,975 mg/kg-day. The authors reported that histological examination did not reveal any significant treatment-related lesions in these tissues. A limited number of endpoints were evaluated in this study, and reproductive organs were not evaluated. This study identifies NOAEL and LOAEL values of 0.1 and 1.0% in the diet (142 and 1,417 mg/kg-day), respectively, for a biologically significant increase in relative liver weight (>25% increase compared with controls).

Table B.3. Body and Organ Weight Data for Weanling Male Albino Rats Exposed to DiBP in the Diet for 1 Month

Endpoint	DiBP Concentration in Diet					
	0	**0.01%**	**0.1%**	**1.0%**	**2.0%**	**5.0%**
Estimated dose (mg/kg-day)	0	15	142	1,417	2,975	8,911
Body weight (g)	226 ± 25[a,b]	189 ± 15	194 ± 26	189 ± 15	181 ± 24	141 ± 14[c]
Absolute organ weights (g)						
Liver	10.2 ± 0.7	8.44 ± 0.7	8.93 ± 1.3	10.7 ± 1.1[c]	11.7 ± 2.1[c]	11.5 ± 1.3[c]
Kidney	1.97 ± 0.3	1.70 ± 0.2	1.76 ± 0.2	1.76 ± 0.2	1.76 ± 0.3	1.51 ± 0.2
Relative organ weights (mg/g-body weight)						
Liver	45.2 ± 2.8	44.7 ± 2.8	47.3 ± 13	56.7 ± 1.7[d]	64.6 ± 4.4[d]	80.9 ± 1.2[d]
Kidney	8.69 ± 0.7	8.98 ± 0.6	9.28 ± 1.9	9.32 ± 0.4	9.73 ± 0.2[d]	10.7 ± 0.5[d]

[a]Values are means ± SD.
[b]SDs were calculated for this review from individual animal data for this study.
[c]Significantly different from low-dose group, $p < 0.05$ (unpaired t-test conducted for this review).
[d]Significantly different from control group, $p < 0.05$ (unpaired t-test conducted for this review).

Source: Hodge (1953).

In the 4-month study, groups of weanling albino rats (5/sex/group) were maintained on a diet containing 0, 0.1, 1.0, or 5.0% DiBP (Hodge, 1954). Using the body weights of the rats in this study as averaged across all 4 months of exposure (259, 267, 244, and 151 g for males and 173, 165, 176, and 144 g for females at 0, 0.1, 1.0, and 5.0%, respectively, as reported in Appendix iii), and assuming reference values for food consumption (0.018 and 0.014 kg/day for male and female rats of an unspecified strain, respectively [U.S. EPA, 1988]), these concentrations correspond to doses of 0, 67, 738, or 5,960 mg/kg-day in males and 0, 85, 795, or 4,861 mg/kg-day in females. Mortality and weekly body weight gain were monitored. Hematology (red blood cell [RBC] count, hemoglobin [Hgb], total and differential WBC count, and numerous RBC characteristics: basophilic stippling, nucleated cells, erythroblasts, poikilocytosis, anisocytosis, and basophilia) was performed on blood samples collected near the end of the exposure period from each rat. At termination, gross necropsy was performed, and organ weights (liver, kidney, testes, lung, brain, stomach, heart, and spleen) were recorded. Histopathology of the liver and kidneys was evaluated in all animals. This study did not appear to evaluate reproductive tissues, and the study authors did not perform statistical analyses.

One control female and one high-dose female died during the study (Hodge, 1954). These deaths were attributed to respiratory infection ("sniffles") and not treatment. Average body weight gain was decreased by >10% in high-dose males and females, and in females exposed to 795 mg/kg-day. Table B.4 summarizes the significant changes in terminal body and liver weights observed in rats maintained on a diet containing DiBP for 4 months. As shown, terminal body weights were significantly lower than controls among high-dose rats (decreased 43% for males and 13% for females) and comparable to controls among mid- and low-dose rats (<10% difference from controls). No significant dose-related changes were observed based on hematology, although occasional elevations in WBC were observed. These changes were attributed to the presence of infection in the colony; a number of cases of sniffles were observed during the study. Absolute and relative liver weights were elevated in high-dose males by 5 and 45%, respectively, and in high-dose females by 29 and 38%, respectively. Pathological examinations of liver tissue were reported to be unremarkable. Both absolute and relative testes weights were markedly reduced in the high-dose group to approximately 30 and 55% of control values, respectively. Gross examination at necropsy revealed an extremely large spleen in one female rat and consistently small testes in the high-dose males. Histopathology of these organs was not performed. No other notable changes were reported. The study identified a LOAEL of 5% DiBP in the diet (5,960 and 4,861 mg/kg-day in males and females, respectively) and a NOAEL of 1% DiBP (738 and 795 mg/kg-day in males and females, respectively) based on changes in body and organ weights. Rats fed diets containing 5% DiBP experienced significant

decreases in body weights (>10% compared with controls) and absolute and relative testes weights (>30% compared with controls) and a significant increase in relative liver weights (>30% compared with controls). Histological examination of liver revealed no exposure-related lesions, and although testes were grossly affected at the highest DiBP exposure dose, this tissue was not examined histologically.

Table B.4. Body and Organ Weight Data for Albino Rats Exposed to DiBP in the Diet for 4 Months

Endpoint	DiBP Concentration in Diet			
	0	0.1%	1.0%	5.0%
Males				
Estimated dose (mg/kg-day)	0	67	738	5,960
Body weight (g)	359 ± 37[a,b]	373 ± 48.1	326 ± 33.4	204 ± 24.1[c]
Absolute organ weights (g)				
Testes	3.06 ± 0.28	3.11 ± 0.28	3.02 ± 0.17	0.93 ± 0.11[c]
Liver	11.7 ± 1.3	12.4 ± 1.6	13.1 ± 2.3	12.3 ± 1.8
Relative organ weights (mg/g-body weight)				
Testes	8.33 ± 1.16	8.40 ± 0.87	9.29 ± 0.64	4.58 ± 0.58[c]
Liver	32.6 ± 2.0	33.2 ± 0.55	39.9 ± 4.2[c]	60.1 ± 3.2[c]
Females				
Estimated dose (mg/kg-day)	0	85	795	4,861
Body weight (g)	216 ± 16.5	214 ± 25.7	231 ± 24.3	187 ± 15.5[c]
Absolute liver weight (g)	7.5 ± 0.61	7.5 ± 0.84	8.6 ± 0.82	10.5 ± 1.2[c]
Relative liver weight (mg/g-body weight)	34.8 ± 3.0	34.9 ± 1.8	37.5 ± 3.4	56.4 ± 2.0[c]

[a]Values are means ± SD.
[b]SDs were calculated for this review from individual animal data for this study.
[c]Significantly different from controls, $p < 0.05$ (unpaired t-test conducted for this review).

Source: Hodge (1954).

Hodge (1954) also evaluated the effects of dietary DiBP (purity not reported) in dogs. In this study, one male and one female dog (type not reported) were fed diets supplemented with DiBP at a daily rate of 0.1 mL/kg feed (male) or 2.0 mL/kg feed (female) for 2 months. Using the density of DiBP (1,049 mg/ml), the average body weights of the dogs in the study (7.8 and 9.8 kg for the male and female, respectively; overall averages based on averages reported in Summary Table 14 by Hodge [1954]), and assuming reference values for food consumption (0.083 and 0.074 kg/day in male and female beagles, respectively, based on a dry diet and subchronic exposure [U.S. EPA, 1988]), these concentrations correspond to doses of 1 and 16 mg/kg-day for the male and female dog, respectively. No concurrent controls were included

in the study. General condition and appetite were monitored throughout the study, and body weights were measured weekly. Urine samples were collected near the start and near the end of the study and were examined for sugar and protein percentages. Blood was sampled from each dog prior to the start of the study and near the end of the study for hematology (RBC, total and differential WBC, Hgb, and numerous RBC characteristics including basophilic stippling, nucleated cells, erythroblasts, poikilocytosis, anisocytosis, and basophilia). An additional blood sample was collected from the male dog midway through the experiment for hematological evaluation. At termination, gross necropsy was performed, and organ weights (liver, kidneys, lungs, brain, heart, and spleen) were recorded. Histopathology was performed on heart, lungs, spleen, gastrointestinal tract, liver, pancreas, adrenals, kidneys, bladder, testis, uterus, ovaries, thyroid, skin (male only), brain (female only), and bone marrow.

Both dogs remained in good general condition throughout the test, maintaining healthy appetites and no signs of illness (Hodge, 1954). During the study, the male dog gained a pound of weight and the female dog lost a half pound of weight. Sugar and protein levels in urine were normal, and no significant hematological changes were observed. Gross pathology was unremarkable. The author reported that aside from elevated relative liver weight in the female dog, the organ weights were within normal ranges. This statement indicates that comparisons were made by the author to historical controls at this laboratory, but no further information on historical controls is reported. Histology revealed very few mature sperm present in the testis of the male dog and signs of chronic infection in the kidney of the female dog. The author reported that the changes seen in the kidney are common in the colony. Liver histology was normal in the female dog, even though the liver was enlarged. The small number of animals tested and the lack of concurrent controls or information on historical controls limit the derivation of an effect level for this study; however, the report provides limited evidence for a possible DiBP effect on the testes in dogs.

Hardin et al. (1987)

As part of a range-finding study, groups of virgin CD-1 (SPF) female mice (50 controls, 10/treatment group) were given DiBP (in corn oil, purity not reported) via gavage doses of 0, 1,795, 3,225, 5,790, or 10,400 (undiluted) mg/kg-day once daily for 8 consecutive days (Hardin et al., 1987; NIOSH, 1983). Mice were observed for clinical signs of toxicity twice daily during treatment and once daily after the 8th day of dosing until they were sacrificed on the 8th day following the final dose. Mice that died during the study were necropsied to exclude dosing error as a cause of death. Body weights were measured on the first and last days of dosing and

4 and 8 days after the final dose. Survival rates at the end of the 8-day dosing period were 100, 100, 100, 50, and 80% at 0, 1,795, 3,225, 5,790, and 10,400 mg/kg-day, respectively. The two deaths at the highest dose were attributed to dosing error. At the end of the 8-day observation period following dosing, survival rates were similar, except for an additional death in the 3,225 mg/kg-day group that was attributed to dosing error. Languidness was observed in mice treated with 3,225 and 5,790 mg/kg-day for a brief period between days 1 and 4 of treatment. Animals in the highest dose group had urine stains on the abdomen and in the anal region at various times during the dosing interval and had rough hair coat near the end of treatment. There were no significant differences in body weights or body weight changes in any group treated with DiBP. As a result, NIOSH (1983) identified a minimum effect dose of 4,000 mg/kg-day for use in the gestational exposure study described below.

Hardin et al. (1987) reported consolidated results for a list of chemicals evaluated using a Chernoff and Kavlock screening assay to evaluate postnatal effects in mice. Results reported for DiBP are based on a study conducted by Hazelton Laboratories America, Inc. (NIOSH, 1983). In this study, groups of 50 timed-pregnant female CD-1 (SPF) mice were given DiBP (in corn oil, purity not reported) via gavage doses of 0 or 4,000 mg/kg-day once daily on GDs 6–13. Mice were observed for clinical signs of toxicity twice daily during treatment and once daily on GDs 14–17. Body weights were measured prior to treatment on GD 6 and again on GD 17. Mice that died during the study period were necropsied to exclude dosing error as a cause of death. Dams were allowed to litter and the numbers and weights of live pups were recorded on PNDs 1 and 3. Maternal body weights on PND 3 were also recorded. Females that failed to deliver by the presumed GD 22 were sacrificed and uteri were examined. At a dose of 4,000 mg/kg-day, 27/50 dams died and none of the pregnant dams gave birth to a live litter. This study identified 4,000 mg/kg-day on GDs 6–13 as a lethal dose for pregnant mice. The range-finding study in virgin mice was apparently inadequate for assessing the effects in pregnant mice, which may be more susceptible to DiBP toxicity.

Singh et al. (1972)

In a comparative study on eight different phthalate esters, Singh et al. (1972) administered DiBP (purity not reported) to groups of five timed-pregnant Sprague-Dawley rats at doses of 0 (untreated), 0.375, 0.75, or 1.25 mL/kg (approximately 389, 779, and 1,298 mg/kg) by i.p. injection on GDs 5, 10, and 15. Additional control groups (5 rats/group) received a similar volume of distilled water, normal saline, or cottonseed oil. Maternal toxicity was not evaluated in this study. Dams were sacrificed on GD 20, 1 day prior to parturition, for examination of

ovaries (count of corpora lutea) and uterine contents (counts of implantation, resorptions, and live and dead fetuses). Fetuses were weighed and examined for gross malformations. Additionally, a randomly selected number of fetuses (between 30 and 50% of the total) were taken for evaluation of skeletal malformations. There was no significant difference in the number of corpora lutea at any dose level in comparison to the untreated controls. Resorptions were higher among rats receiving 1,298 mg/kg (25.8%), compared with zero resorptions among the untreated controls. At 779 mg/kg, 2/52 fetuses were found dead, but no fetuses were found dead at the low or high dose. Fetal weights were significantly lower than untreated controls at all dose levels (25, 28, and 59% less than controls at 389, 779, and 1,298 mg/kg, respectively). Two fetuses at 779 mg/kg were without eyes due to incomplete formation of the head. A dose-related increase in skeletal abnormalities (partially elongated and fused ribs) was observed. No gross or skeletal abnormalities were observed among the untreated controls. The study authors did not identify developmental effect levels. The reported results were inadequate to determine maternal toxicity, but were sufficient to determine that 389 mg/kg (the lowest i.p. dose administered on GDs 5, 10, and 15) was a LOAEL for decreased fetal body weight.

BASF (2003)

In an unpublished study submitted by BASF (2003) to the European Commission (2004), DiBP (purity not reported) was administered to groups of pregnant Wistar rats (25 females/) in the diet at 0, 88, 363, or 942 mg/kg-day on GDs 6–20. The study was performed under the Organisation for Economic Co-operation and Development (OECD) guidelines. Maternal endpoints were food consumption and body weight gain. Developmental toxicity endpoints appeared to include conception rate, numbers of corpora lutea, implantations, resorptions and viable fetuses, sex ratio, fetal body weight, and evaluation of external, visceral, and skeletal malformations and variations. Data were not shown. However, results summarized by the European Commission (2004) indicate that maternal toxicity was observed at the highest dose, characterized by decreased food intake and overall body weight gain (about 11% below controls and 25% for corrected body weight gain). No effect was observed on conception rate or number of corpora lutea, implantations, resorptions, or viable fetuses. There was no effect on sex ratio. Fetal body weights were decreased at the highest dose (about 5% below controls). No significant increases in the incidence of any external, soft-tissue, or skeletal malformations were observed. A significant increase in the incidence of some skeletal variations was observed at the high dose, including incomplete ossification of sternebrae (38.4% of affected fetuses per litter in controls, 42.7% in the low-dose group, 46.5% in the mid-dose group, and 68.7% in the high-dose group, $p < 0.01$) and unilateral ossification of sternebrae (0.0% of affected fetuses per litter in controls,

low-dose, and mid-dose groups, and 4.3% in the high-dose group, $p < 0.05$). The incidences of incomplete ossification were apparently within the range of historical controls, while the incidence of unilateral ossification of sternebrae in the high-dose group was outside the range of historical controls (data not provided). The secondary source reported that this study did not provide data on male reproductive system parameters. This study identified a LOAEL of 942 mg/kg-day and a NOAEL of 363 mg/kg-day for maternal toxicity in pregnant Wistar rats based on decreased gestational weight gain and for developmental toxicity based on decreased fetal body weights and incomplete ossification of sternebrae.

Howdeshell et al. (2008)

Howdeshell et al. (2008) investigated the effects of oral exposure to five phthalate esters (DiBP, dibutyl phthalate, benzylbutyl phthalate, diethylhexyl phthalate, and dipentyl phthalate [DPP]) individually and as a mixture in rats. In the individual chemical study, groups of 5–8 pregnant Sprague-Dawley rats were given DiBP (99% pure, in corn oil) via gavage doses of 0, 100, 300, 600, or 900 mg/kg-day on GDs 8–18. Maternal evaluations were limited to mortality, signs of toxicity, and daily body weights. At the end of exposure, the dams were sacrificed for examination of uterine contents (counts of implantations, resorptions, and live and dead fetuses). Fetuses were sacrificed, and testes were removed from the first three males for assessment of ex vivo testosterone production. The litter was used as the statistical unit for analysis of data in offspring.

Effects related to DiBP treatment are shown in Table B.5 (Howdeshell et al., 2008). Data are presented as litter means. Among the eight dams in the 300 mg/kg-day group, one female was not pregnant and two either died or were removed from the study due to dosing errors. Dams exposed to DiBP alone at 900 mg/kg-day exhibited reduced body weight gain (42% lower than controls). DiBP induced complete litter loss in one dam at 900 mg/kg-day and induced >50% resorptions in two dams at 900 mg/kg-day and one dam at 600 mg/kg-day. On average, a higher number of resorptions (40-fold higher than controls) and fewer live fetuses (62% less than controls) were observed at 900 mg/kg-day DiBP. Fetal testicular testosterone production was significantly reduced in a dose-related manner at ≥300 mg/kg-day (from 40% lower than controls at 300 mg/kg-day to 63% lower at 900 mg/kg-day). The study authors did not identify effect levels, but did estimate ED_{50} values from sigmoidal regression models of the dose-response data for effects on testosterone production; the ED_{50} estimated for DiBP was 440 ± 16 mg/kg-day. These results indicate maternal NOAEL and LOAEL values of 600 and 900 mg/kg-day, respectively, for reductions in body weight gain, and developmental NOAEL and LOAEL values

of 100 and 300 mg/kg-day, respectively, for decreased fetal testicular testosterone production. Increased numbers of resorptions and decreased numbers of live fetuses were observed at higher doses.

Table B.5. Maternal and Fetal Effects in Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 8–18

Endpoint	Dose (mg/kg-day)				
	0	**100**	**300**	**600**	**900**
Maternal Effects					
Number of pregnant females on GD 18	5/5[a] (100%)	8/8 (100%)	5/8 (63%)	5/5 (100%)	5/5 (100%)
Number of pregnant females with whole litter loss	0/5	0/8	0/5	0/5	1/5
Body weight (g) GD 8 GD 18	 300.1 ± 1.7[b] 373.5 ± 4.3	 294.8 ± 12.0 374.7 ± 6.7	 290.6 ± 16.6 366.8 ± 10.0	 299.3 ± 2.2 347.2 ± 6.5[c]	 301.7 ± 3.1 344.6 ± 6.2[c]
Body weight gain (g)	73.4 ± 4.3	79.9 ± 10.2	76.2 ± 14.8	47.9 ± 5.6	42.9 ± 5.5[c]
Fetal Effects					
Number of implantations[d,e]	13.7 ± 0.9 (3)	14.8 ± 0.8 (4)	16.0 ± 1.0 (3)	12.7 ± 1.2 (3)	13.3 ± 0.9
Number of live fetuses[e]	13.3 ± 0.7 (3)	13.5 ± 0.5 (4)	15.3 ± 1.5 (3)	9.3 ± 2.6 (3)	5.0 ± 3.6[c] (3)
Total resorptions	0.2 ± 0.2	1.0 ± 0.5	0.4 ± 0.4	2.0 ± 1.1	7.8 ± 2.5[c]
Fetal mortality (%)[f,e]	1.3 ± 1.3 (3)	4.6 ± 2.6 (4)	2.7 ± 2.7 (3)	17.2 ± 10.4	59.0 ± 30.2[c]
Testicular testosterone production on GD 18 (ng/testis/3 hour)[g]	5.7 ± 0.13 (15/5)	5.44 ± 0.19 (24/8)	3.40 ± 0.28[c] (15/5)	2.31 ± 0.35[c] (15/5)	2.09 ± 0.91[c] (6/2)

[a]Number observed/total tested.
[b]Values are means ± SE.
[c]Significantly different from controls, $p < 0.05$.
[d]Implantations = live fetuses + dead fetuses + total resorptions.
[e]Number in parentheses indicates number of litters in the analysis when different from number of dams on GD 18.
[f]Fetal mortality = ([resorptions + dead fetuses] / implantations) × 100.
[g]Numbers in parentheses indicate number of individual fetuses examined / number of litters examined.

Source: Howdeshell et al. (2008).

Saillenfait et al. (2008, 2006, 2005)

Saillenfait and colleagues investigated the developmental toxic effects of DiBP in Sprague-Dawley rats (Saillenfait et al., 2008, 2006, 2005). In a dose range-finding study reported as an abstract, DiBP (in olive oil, purity not reported) was administered via gavage to groups of 10–14 timed-pregnant Sprague-Dawley rats at 0, 250, 500, 750, or 1,000 mg/kg-day on

GDs 6–20 (Saillenfait et al., 2005). Maternal evaluations were limited to mortality, signs of toxicity, and body weight (frequency of measurement not reported). At the end of exposure, the dams were sacrificed for examination of uterine contents (counts of implantations, resorptions, and live and dead fetuses). All live fetuses were weighed and examined externally. Fetuses were sacrificed and sexed, and internal gross examination of the reproductive tract was performed.

No mention of maternal deaths or overt signs of toxicity was made by the authors. Results are summarized in Table B.6. Dams exposed to DiBP at doses ≥750 mg/kg-day experienced a significant reduction in body weight gain (31–38% lower than controls). However, after correcting for gravid uterine weights, maternal weight gains during the entire exposure period were not significantly different from controls among any DiBP dose group. Significantly more resorptions occurred among dams exposed to DiBP at doses ≥500 mg/kg-day (4–24-fold higher than controls). Numbers of live fetuses and fetal weight were significantly reduced at ≥750 mg/kg-day (39–57% fewer live fetuses than controls and fetal weights 16–21% lower than controls). External examination revealed malformations among two fetuses from two different litters at 750 mg/kg-day that consisted of general edema in one and multiple malformations including anal atresia, small genital tubercle, ectrodactyly, and shortened hindlimbs in the other. However, these findings cannot be conclusively attributed to DiBP treatment based on the limited incidence and absence of a dose-response pattern. Internal examination revealed undescended testes in 56 and 70% of the male fetuses at 750 and 1,000 mg/kg-day, respectively; undescended testes were not found in fetuses in the control, 250, or 500 mg/kg-day groups. No significant difference in fetal sex ratio was observed. These results indicate maternal NOAEL and LOAEL values of 500 and 750 mg/kg-day, respectively, for reductions in body weight gain, and developmental NOAEL and LOAEL values of 250 and 500 mg/kg-day, respectively, for increased incidence of resorptions. Testicular effects in male fetuses (increased incidence of litters with fetuses with undescended testes) resulted in NOAEL and LOAEL values of 500 and 750 mg/kg-day, respectively.

Table B.6. Maternal, Reproductive, and Fetal Findings in Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 6–20

Endpoint	Dose (mg/kg-day)				
	0	250	500	750	1,000
Maternal Effects					
Number of pregnant rats	11	10	11	14	12
Maternal body weight gain GDs 6–21 (g)	152 ± 23[a]	155 ± 22	149 ± 28	105 ± 47[b]	94 ± 41[b]
Corrected weight gain[c]	51 ± 19	55 ± 11	55 ± 12	46 ± 13	53 ± 17
Fetal Effects					
Number of implantations/litter	13.5 ± 2.1	13.4 ± 2.9	14.0 ± 3.6	13.0 ± 3.0	12.6 ± 5.5
Percentage of resorptions/litter	2.5 ± 8.2	4.2 ± 6.9	8.8 ± 10.4[d]	38.3 ± 34.4[d]	61.2 ± 31.2[d]
Number of live fetuses/litter	13.2 ± 2.6	12.8 ± 2.9	12.6 ± 3.5	8.1 ± 5.2[b]	5.7 ± 5.5[b]
Fetal body weight (g)	5.6 ± 0.1	5.8 ± 0.3	5.4 ± 0.4	4.7 ± 0.6[b]	4.4 ± 0.6[b]
Percentage of male fetus/litter	47.4 ± 14.0	51.3 ± 19.7	50.5 ± 15.2	45.6 ± 21.0	58.6 ± 25.3
Incidence of male fetuses (litters) with undescended testes	0/70 (0/11)	0/68 (0/10)	0/68 (0/11)	31/55 (9/14)	26/37 (7/12)

[a]Values are means ± SD.
[b]Significantly different from controls, $p < 0.05$ (Dunnett's test).
[c]Body weight gain during GDs 6–21 minus gravid uterine weight.
[d]Significantly different from controls, $p < 0.05$ (Kruskal Wallis, Mann-Whitney test).

Source: Saillenfait et al. (2005).

Based on the results from the range-finding study described above, Saillenfait et al. (2006) exposed groups of 20–22 timed-pregnant Sprague-Dawley rats to DiBP (≥99% pure, in olive oil) via gavage doses of 0, 250, 500, 750, or 1,000 mg/kg-day on GDs 6–20. Maternal evaluations included daily monitoring for mortality and signs of toxicity and food consumption and body weight measurements at 3-day intervals. At the end of exposure, the dams were sacrificed for examination of uterine contents (counts of implantations, resorptions, and live and dead fetuses). The number of corpora lutea in each ovary was also recorded. Uteri without visible implantation sites were further evaluated for detection of very early resorptions. All live fetuses were weighed and examined externally. Fetuses were sacrificed; half were subjected to visceral examination and half were subjected to skeletal examination. All fetuses were sexed, and males were evaluated for degree of TTM. An increasing value of TTM indicates decreased descendence of the testes during development.

Maternal and fetal effects are summarized in Tables B.7, B.8, and B.9 (Saillenfait et al., 2006). There were no maternal deaths or overt signs of maternal toxicity in any of the groups. Pregnancy rates were reported to be between 83 and 92% for controls and DiBP-exposed groups.

Dams exposed to DiBP at doses of ≥500 mg/kg-day gained significantly less weight than controls at the beginning (GDs 6–9) and near the end (GDs 15–18) of treatment. Changes in body weight were also significantly different from controls among high-dose rats at the end of treatment (GDs 18–21) and when evaluated over the course of the entire exposure period (GDs 6–21). However, after correcting for gravid uterine weights, maternal weight gains over the course of the exposure period were not significantly different from controls among any dose group. Maternal food consumption was comparable across groups. Although there was no apparent effect of DiBP treatment on the number of implantations, there was a marked increase in resorptions (four- to ninefold higher than controls) and a significant reduction in the number of live fetuses (21–52% fewer than controls) at ≥750 mg/kg-day.

Table B.7. Body and Uterine Weights in Pregnant Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 6–20

Endpoint	Dose (mg/kg-day)				
	0	**250**	**500**	**750**	**1,000**
Maternal Effects					
Initial body weight (g)					
GD 0	222 ± 12[a]	225 ± 14	225 ± 17	226 ± 13	224 ± 15
Body weight gain (g)					
GDs 0–6	29 ± 6	29 ± 9	28 ± 8	30 ± 6	28 ± 7
GDs 6–9	12 ± 4	12 ± 4	9 ± 5[b]	8 ± 5[b]	8 ± 3[b]
GDs 9–12	18 ± 3	16 ± 4	16 ± 4	15 ± 4	16 ± 5
GDs 12–15	19 ± 4	20 ± 6	18 ± 9	19 ± 5	16 ± 4
GDs 15–18	39 ± 6	39 ± 13	32 ± 10[b]	32 ± 9[b]	20 ± 9[b]
GDs 18–21	47 ± 8	46 ± 15	42 ± 15	41 ± 11	23 ± 14[b]
GDs 6–21	135 ± 16	133 ± 34	116 ± 32	116 ± 25	83 ± 28[b]
Gravid uterine weight (g)	98 ± 17	96 ± 30	79 ± 29[b]	71 ± 19[b]	38 ± 23[b]
Corrected weight gain[c]	37 ± 8	37 ± 13	37 ± 15	44 ± 10	44 ± 11

[a]Values are means ± SD.
[b]Significantly different from controls mean, $p < 0.05$.
[c]Body weight gain during GDs 6–21 minus gravid uterine weight.

Source: Saillenfait et al. (2006).

Table B.8. Implantation, Fetal Survival, and Fetal Body Weight Data for Pregnant Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 6–20

Endpoint	Dose (mg/kg-day)				
	0	**250**	**500**	**750**	**1,000**
Number of live litters[a]	22/22	21/22	21/22	21/21	18/20
Number of implantation sites/litter[b]	13.6 ± 2.0	13.6 ± 2.9	12.1 ± 4.5	14.2 ± 2.4	13.5 ± 2.9
Percentage of postimplantation loss/litter[b]	6.7 ± 7.6	11.0 ± 23.6	13.9 ± 20.9	28.2 ± 18.9[c]	59.6 ± 21.5[c]
Percentage of dead fetuses/litter[b]	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 1.5	0.7 ± 2.1	0.3 ± 1.5
Percentage of resorptions/litter[b]	6.7 ± 7.6	11.0 ± 23.6	13.6 ± 20.8	27.6 ± 18.2[c]	59.3 ± 22.2[c]
Number of live fetuses/litter[b]	12.8 ± 2.4	13.1 ± 3.1	11.3 ± 3.5	10.1 ± 3.1[c]	6.2 ± 3.2[c]
Percentage of male fetuses/litter[b]	52.4 ± 14.0	50.5 ± 11.1	49.6 ± 19.4	52.6 ± 16.7	61.5 ± 19.0
Fetal body weight (g)					
All[b]	5.71 ± 0.28	5.69 ± 0.33	5.31 ± 0.40[c]	4.72 ± 0.33[c]	4.32 ± 0.35[c]
Male[b]	5.84 ± 0.31	5.85 ± 0.34	5.49 ± 0.48[c]	4.85 ± 0.32[c]	4.39 ± 0.43[c]
Female[b]	5.57 ± 0.29	5.52 ± 0.34	5.10 ± 0.33[c]	4.57 ± 0.4[c]	4.14 ± 0.35[c]

[a]Live litters/total litters including pregnant females at euthanization.
[b]Values are means ± SD.
[c]Significantly different from controls mean, $p < 0.05$.

Source: Saillenfait et al. (2006).

Table B.9. Malformations and Variations in Fetuses of Pregnant Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 6–20

Endpoint	Dose (mg/kg-day) 0	250	500	750	1,000
Number of fetuses (litters) examined					
External	281 (22)	276 (21)	237 (21)	212 (21)	111 (18)
Visceral	141 (22)	138 (21)	119 (21)	106 (21)	56 (18)
Skeletal	140 (22)	138 (22)	118 (22)	106 (21)	55 (18)
Malformations					
Number of fetuses (%) with malformations					
External[a]	0	0	0	5 (2.4)[d]	6 (5.4)[d]
Visceral[b]	0	2 (1.4)	2 (1.7)	13 (12.3)[d]	10 (17.9)[d]
Skeletal[c]	0	0	4 (3.4)	18 (17.0)[d]	34 (61.8)[d]
Number of litters (%) with malformations					
External[a]	0	0	0	4 (19.0)	4 (22.2)
Visceral[b]	0	1 (4.8)	2 (9.5)	8 (38.1)[d]	8 (44.4)[d]
Skeletal[c]	0	0	4 (19.0)	11 (52.4)[d]	15 (83.3)[d]
Mean % fetuses with malformations/litter					
External[a]	0	0	0	2.3 ± 5.1	3.7 ± 7.7
Visceral[b]	0	1.2 ± 5.5	1.7 ± 5.5	13.1 ± 23.9[e]	15.8 ± 20.9[e]
Skeletal[c]	0	0	3.2 ± 6.9	18.3 ± 22.8[e]	67.1 ± 37.1[e]
Number of fetuses (litters) with fused or fused and scrambled sternebrae	0	0	0	12 (7)[e]	26 (13)[e]
Variations					
Number of fetuses (litters) with visceral variations					
Ectopic testes[f]	0	0	3 (2)	30 (16)[e]	30 (16)[e]
TTM[g]	2.6 ± 3.6	3.8 ± 3.3	13.6 ± 11.0[e]	42.2 ± 11.8[e]	58.1 ± 12.8[e]
Number of fetuses (litters) with skeletal variations					
Sternebrae, fused first and second	1 (1)	0	8 (4)	29 (11)[e]	5 (4)
Ribs					
Cervical, rudimentary	0	0	2 (2)	12 (9)[e]	9 (6)
14th, any supernumerary	23 (11)	32 (14)	42 (18)	72 (20)[e]	52 (18)[e]
14th, long supernumerary	1 (1)	1 (1)	2 (2)	15 (9)[e]	9 (9)[e]
Thoracic or lumbar vertebral centra, incomplete ossification	3 (2)	8 (6)	7 (7)	18 (14)[e]	16 (8)[e]

[a]Incidences of fetuses or litters with individual external malformations in exposed groups were not significantly ($p < 0.05$) different from control values. External malformations observed in more than one fetus were exencephaly and meningoencephalocele.
[b]Incidences of fetuses or litters with individual visceral malformations in exposed groups were not significantly ($p < 0.05$) different from control values. Visceral malformations observed in more than one fetus were anophthalmia, transposed aorta or pulmonary artery, and absent kidney and ureter.
[c]Incidences of fetuses or litters with individual skeletal malformations in exposed groups were not significantly ($p < 0.05$) different from control values, except for incidences of fetuses or litters with fused or fused and scrambled sternebrae. Other skeletal malformations observed in more than one fetus were cleft sternum, fused ribs, fused cervical archs, fused or misaligned thoracic or lumbar centra, and hemicentric thoracic or lumbar centrum.
[d]Significant difference from the vehicle control, $p < 0.05$ (Fisher's test).
[e]Significant difference from the vehicle control, $p < 0.05$ (Mann-Whitney test).
[f]Testes outside the normal pathway of descent.
[g]Degree of TTM: (Distance between the bladder neck and the lower pole of the testis) / (distance between the lower pole of the kidney and the bladder neck) × 100. The value of TTM increases with degree of inhibition of normal descent of testes during development.

Source: Saillenfait et al. (2006).

Exposures to ≥500 mg/kg-day DiBP produced a variety of effects on the fetus (Saillenfait et al., 2006). At ≥500 mg/kg-day, fetal body weights (males and females) were significantly lower than controls (reduced by 7, 17, and 24% relative to controls at 500, 750, and 1,000 mg/kg-day, respectively, $p < 0.05$). In addition, the incidences of external, visceral, and skeletal malformations were increased after exposure to ≥750 mg/kg-day. Aside from a significant increase in the incidence of fused sternebrae, there were no significant differences in the incidence of any specific malformations when considered individually. However, the incidences based on total number of fetuses with external malformations and total numbers of fetuses or litters with visceral or skeletal malformations were significantly higher than controls. The most common malformations observed were anterior neural tube closure defects, anophthalmia, urinary tract and vascular defects, and defects of the axial skeleton, including fused vertebral arch or centrum, hemicentrum, and sternal abnormalities. Visceral and skeletal variations were increased as well. There were significant increases in the incidences of undescended testes and supernumary ribs in groups exposed to ≥750 mg/kg-day. Additionally, in groups exposed to ≥500 mg/kg-day, the degree of TTM in relation to the bladder was significantly increased over that of controls. In fact, at the highest dose, about two-thirds of the testes were located in the upper half of the abdominal cavity.

This study identified a LOAEL of 500 mg/kg-day and a NOAEL of 250 mg/kg-day for maternal toxicity based on significantly decreased gravid uterine weights and for developmental toxicity based on reduced fetal growth and increased degree of TTM. Male fetuses with ectopic (undescended) testis appeared at the LOAEL, and their incidence increased with dose. Increased incidences of resorptions and developmental malformations were seen at higher doses (≥750 mg/kg-day).

Saillenfait et al. (2008) conducted a follow-up study to further evaluate the postnatal effects of in utero exposure to DiBP on male reproductive development. DiBP (>99% pure in olive oil) was administered via gavage to groups of 11–13 timed-pregnant Sprague-Dawley rats at 0, 125, 250, 500, or 625 mg/kg-day on GDs 12–21. This period of gestation is considered a sensitive time for male reproductive tract differentiation in rats. The authors reported that the doses used in this study were based on an unpublished preliminary study in which exposure to DiBP at 625 mg/kg-day on GDs 12–21 caused reproductive tract malformations in male offspring without affecting litter size or pup survival. Changes in maternal weight were monitored every 3rd day during the treatment period and on days 1, 4, 7, 14, and 21 postpartum. After weaning, nursing dams were sacrificed and evaluated for the number of implantation sites.

Litters were examined immediately following birth to determine the number of viable and stillborn pups, and AGD was measured on PND 1. Litters were culled to 10 pups on PND 4, retaining as many males as possible. At weaning on PND 21, three or four males from each litter were randomly selected for further postnatal assessment. Confirmation of sex was performed on discarded pups throughout this period. Individual pup body weight was recorded on PNDs 1, 4, 7, 14, and 21 and then at weekly intervals until study termination. All retained males were examined for PPS from PND 40 until the prepuce was completely retracted from the glans penis. Adult males were sacrificed and examined externally on either PNDs 76–86 (PNWs 11–12, two males in each litter) or PNDs 111–122 (PNWs 16–17, the remaining males in each litter). Testes, epididymides, seminal vesicles (with the coagulating glands and seminal fluid), and prostates were weighed. Histopathology was performed on the testes and epididymides of all DiBP animals necropsied on PNWs 11–12.

No significant signs of maternal toxicity following exposure to DiBP during gestation or lactation were observed (Saillenfait et al., 2008). Gestation length, postimplantation loss, percentage of pups born alive, number of live pups per litter, and pup survival to PNDs 4 and 21 were not significantly different between DiBP groups and controls. However, significant effects on male offspring were observed at DiBP doses of ≥250 mg/kg-day (summarized in Table B.10). At these dose levels, a significant dose-response trend was observed based on reduced male AGD on PND 1. No effect was observed on female AGD. Body weights for both sexes were significantly lower among the 625 mg/kg-day group on PND 1 and remained slightly lower (not statistically significant) than controls during the lactation period. At weaning (PND 21), male body weights of this group were significantly lower than controls. Puberty was delayed in male offspring at ≥500 mg/kg-day. Low-dose males experienced an earlier onset of PPS than controls accompanied by a lower body weight. The authors postulate that this observation was likely due to biological variations and was not considered related to treatment.

Table B.10. Birth Weight, Postnatal Survival, and Early Reproductive Organ Development Endpoints in Male Offspring of Pregnant Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 12–21

Endpoint	Dose (mg/kg-day)				
	0	125	250	500	625
Pup survival PNDs 1–4 (%)	99.4 ± 2.3[a]	98.9 ± 3.8	98.9 ± 2.7	98.2 ± 6.1	92.5 ± 12.0
Pup survival PNDs 4–21 (%)	92.5 ± 8.7	94.0 ± 7.0	97.1 ± 4.7	95.0 ± 9.0	97.0 ± 4.8
Male pup weight PND 1 (g)	7.19 ± 0.71	7.10 ± 0.70	7.04 ± 0.43	7.03 ± 0.53	6.45 ± 0.60[b]
Male AGD PND 1 (mm)	2.55 ± 0.17	2.44 ± 0.15	2.28 ± 0.30[b]	2.02 ± 0.13[b]	1.98 ± 0.16[b]
PPS in male offspring[c]					
Number of males examined (litters)	46 (12)	40 (10)	55 (14)	39 (11)	17 (7)
Age at PPS (d)[d]	46.9 ± 1.5	45.1 ± 1.6[b]	46.3 ± 1.8	51.5 ± 3.1[b]	49.8 ± 3.2[b]
Body weight at PPS (g)	215 ± 11	197 ± 15[b]	205 ± 9b	230 ± 22	220 ± 19

[a]Values are mean ± SD.
[b]Significantly different from controls, $p < 0.05$ (Mann-Whitney test).
[c]PPS describes the separation of the prepuce from the glans penis.
[d]PPS was not evaluated in males with hypospadias (i.e., 5/4 and 22/9 males/litters at 500 mg DiBP/kg-day and 625 mg DiBP/kg-day, respectively).

Source: Saillenfait et al. (2008).

Table B.11 summarizes the findings reported by Saillenfait et al. (2008) observed during the postweaning period. As shown, litter mean pup weights were significantly lower than controls at ≥500 mg/kg-day (7–11% lower than controls). On PNWs 11–12 and 16–17, absolute and relative testes, epididymides, seminal vesicles, and prostate weights were significantly ($p < 0.05$) lower than controls at ≥500 mg/kg-day (Table B.11 shows group mean values for PNWs 16–17, which are representative of the changes in these variables at PNWs 11–12). Absolute prostate weight was also significantly smaller than controls on PNWs 11–12 at 250 mg/kg-day (11% less than controls, $p < 0.05$) and on PNWs 16–17 at 125 mg/kg-day (14% less than controls, $p < 0.05$), but there was no significant difference at these doses when adjusted for body weight. Thoracic areolas and/or nipples were not observed in controls or males from the 125 mg/kg-day group on PNDs 12–14 or at adult necropsy, but were apparent in males at ≥250 mg/kg-day (four to eight pups from two to three litters), increasing in incidence with dose. Mature males displayed severe malformations of the external and internal genitalia at ≥500 mg/kg-day (see Table B.11). The most prevalent malformations among mature males were hypospadias, or defects of the urethra where the urinary opening is abnormally placed (11 and 56% of males at 500 and 625 mg/kg-day, respectively). The more severely affected animals also demonstrated exposed os penis and nonscrotal testis (undescended testis), and nearly half of the high-dose males showing hypospadia also had a cleft prepuce. Additionally, two males from two

different litters displayed blind perineal vaginal pouches, and four males from three litters had a small penis. Markedly underdeveloped or absent testis and/or epididymis were seen in 2 (one male), 16 (seven males from five litters), and 13% (five males from four litters) of males at 250, 500, and 625 mg/kg-day, respectively. One 500 mg/kg-day rat had no prostate and malformed seminal vesicles, and two 625 mg/kg-day rats displayed unilaterally small seminal vesicles. Histology revealed unilateral or bilateral lesions of the seminiferous tubules in all DiBP-treated groups. These lesions increased in severity with dose and were associated with oligospermia or total azoospermia in the corresponding epididymides. Nearly all of the high-dose animals (18/20) demonstrated a complete loss of germ cells. Tubular necrosis was present in three males from the 500 mg/kg-day group and five males from the 625 mg/kg-day group, while "total tubular necrosis was observed in testes dramatically reduced in size (unilateral)" among one and four males from these groups, respectively. Sporadic inflammation of the epididymides and an apparent increase in the number of rats showing Leydig's interstitial cell hyperplasia were observed at ≥500 mg/kg-day. Only slight and unilateral atrophy of seminiferous tubules was noted in 8% of the controls. The study authors noted that histological changes in the seminiferous tubules of rats at the lowest dose tested were observed in males that arose from the same litter.

Table B.11. Body and Reproductive Organ Weights and Reproductive Organ Lesions of Adult Male Offspring of Pregnant Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 12–21

Endpoint	Dose (mg/kg-day)				
	0	125	250	500	625
Weights (g) at PNWs 16–17					
Number of males (litters)	22 (12)	20 (10)	27 (14)	22 (11)	18 (10)
Body weight[a]	476 ± 58	449 ± 53	452 ± 43	424 ± 44[b]	423 ± 58[b]
Right testis[a]	1.93 ± 0.16	1.80 ± 0.48	1.88 ± 0.28	1.62 ± 0.62[c]	0.98 ± 0.76[b,c]
Right epididymis[a]	0.64 ± 0.06	0.59 ± 0.10	0.60 ± 0.06	0.45 ± 0.18[b]	0.36 ± 0.18[b]
Left testis[a]	1.93 ± 0.17	1.76 ± 0.44	1.90 ± 0.28	1.84 ± 0.31[c]	1.17 ± 0.83[b,c]
Left epididymis[a]	0.63 ± 0.06	0.58 ± 0.10	0.60 ± 0.06	0.52 ± 0.13[b]	0.37 ± 0.16[b]
Seminal vesicles[a]	1.90 ± 0.29	1.86 ± 0.27	1.76 ± 0.20	1.45 ± 0.25[b]	1.27 ± 0.41[b]
Prostate[a]	1.10 ± 0.23	0.95 ± 0.20[b]	1.0 ± 0.13	0.91 ± 0.13[b]	0.79 ± 0.23[b]
Thoracic areolas/nipples in male offspring (incidence)					
PNDs 12–14	0/76	0/78	8/96	47/79	56/76
PNWs 11–12 and 16–17	0/46	0/40	4/55	24/44	29/38

Table B.11. Body and Reproductive Organ Weights and Reproductive Organ Lesions of Adult Male Offspring of Pregnant Sprague-Dawley Rats Exposed to DiBP by Gavage on GDs 12–21

Endpoint	Dose (mg/kg-day)				
	0	**125**	**250**	**500**	**625**
External malformations at PNWs 11–12 and 16–17					
Number of adults examined	46	40	55	44	39
Hypospadias	0	0	0	5	22
Exposed os penis	0	0	0	4	11
Cleft prepuce	0	0	0	0	10
Nonscrotal testes	0	0	0	11	30
Histopathology (PNWs 11–12)					
Number males/litters examined[d]	24/12	20/10[e]	28/14[e]	22/11	20/10
Epididymides:					
Oligospermia	0	1	3	2[f]	1[f]
Azoospermia	0	1	3	10[f]	18[f]
Granulomatous inflammation	0	0	0	4	3
Testes: tubular degeneration-atrophy/hypoplasia[g]	2	2	7	16	20
Grade 1 (<5% tubules affected)	2	0	1	3	1
Grade 2 (5–25%)	0	1	1	1	0
Grade 3 (26–45%)	0	0	2	0	2
Grade 4 (46–85%)	0	0	1	4[h]	0
Grade 5 (>85%)	0	1	2	8[h]	17
Testes: tubular necrosis	0	0	1	3	5
Testes: interstitial cell hyperplasia	0	0	0	1	9

[a]Values are mean ± SD. Severely underdeveloped testis and/or epididymis were not included in organ weight means.
[b]Significantly different from controls, $p < 0.05$ (Mann-Whitney test).
[c]When only descended testes were included, the means at 500 mg DiBP/kg-day are 1.77 ± 0.46 (right, n=17) and 1.90 ± 0.16 (left, n=19), and the means at 625 mg DiBP/kg-day are 1.83 ± 0.48 (right, n=7) and 1.87 ± 0.46 (left, n=8); no nonscrotal testis in control.
[d]Two males in each litter.
[e]Histological examination was also performed on two 125 mg DiBP/kg-day males and one 250 mg DiBP/kg-day male, which showed small testes (bilateral) at necropsy on PNWs 16–17. All three had severe degeneration of seminiferous tubules (grade 5), together with oligospermia or azoospermia. These data are not included in the table. At 125 mg DiBP/kg-day, all affected rats were from the same litter.
[f]One male at 500 mg DiBP/kg-day and three males at 625 mg DiBP/kg-day showed azoospermia in one epididymis and oligospermia in the other one. Only azoospermia is mentioned.
[g]Only the highest severity of tubular degeneration-atrophy/hypoplasia was mentioned when the lesion was bilateral; severity based on the approximate percentage of affected semiferous tubules.
[h]In this dose group, 6 of the 12 males with grade 4 or 5 tubular degeneration had descended testes.

Source: Saillenfait et al. (2008).

The results from the Saillenfait et al. (2008) study indicate that the highest dose level, 625 mg/kg-day, was a NOAEL for maternal toxicity in pregnant Sprague-Dawley rats. For effects on male offspring reproductive tissues (Tables B.10 and B.11), the results indicate a

NOAEL of 125 mg/kg-day and a LOAEL of 250 mg/kg-day. Statistically and/or biologically significant effects observed at 250 mg/kg-day were a decrease in male AGD at PND 1, increased thoracic areolas and/or nipples in male offspring, and increased incidence of male offspring at PNWs 16–17 with testicular tubular degeneration-atrophy/hypoplasia. At higher dose levels, the incidence and severity of these effects increased. Other male reproductive tissue effects observed at the two highest dose levels included decreased weights of testes and epididymis at PNWs 11–12 and 16–17 and increased incidence of males with external malformations, including hypospadias, exposed os penis, nonscrotal testes, and azospermia.

Boberg et al. (2008); Borch et al. (2006)

In an evaluation of the ability of DiBP to interfere with male reproductive tract development in rats, groups of eight timed-pregnant Wistar rats (HanTac:WH) were given DiBP (99% pure, in corn oil) via gavage doses of 0 or 600 mg/kg-day from GD 7 to the time of autopsy (Boberg et al., 2008; Borch et al., 2006). One control group and one dose group were autopsied on GD 19 and another control group and dose group were autopsied on GD 21. However, due to a laboratory mix-up, some dams in the latter groups were sacrificed 1 day earlier in pregnancy. Therefore, one-fourth of the fetuses autopsied at "GD 21" were actually only 20 days in gestation (these groups were referred to as "GD 20/21"). Dams were inspected twice daily for general signs of toxicity. At the day of autopsy, dams were euthanized and fetuses were removed. Fetuses were evaluated for body weights, AGD, ovarian estradiol levels, testicular testosterone levels, and testicular histopathology (one or two testes per litter) (Borch et al., 2006). Plasma levels of leptin and insulin were measured in blood collected from fetuses at GD 21 (Boberg et al., 2008). Testes from one or two male fetuses per litter were removed and subjected to gene expression analysis on genes involved in steroid synthesis (16 factors evaluated using real-time polymerase chain reaction) (Boberg et al., 2008). Protein expression levels were evaluated by immunohistochemistry on sections of testis incubated with rabbit polyclonal antibodies. The expression of a peroxisome proliferator activated receptor (levels of PPARα mRNA) was also measured in testes and liver of male fetuses (Boberg et al., 2008).

DiBP treatment did not significantly affect maternal weight gain during pregnancy, litter size, fetal viability, or number of resorptions (data not shown) (Borch et al., 2006). Fetal body weights among the DiBP-exposed groups were lower than controls (≥10%) (Boberg et al., 2008; Borch et al., 2006). However, the differences in fetal body weights between exposed groups and controls were only statistically significantly at GD 19 ($p < 0.05$, data shown graphically). Based on these findings, the study authors included the cubic root of bodyweight as a covariate in the

statistical analysis of AGD. When accounting for body weight, male fetuses from DiBP-dosed dams had significantly lower AGD values than controls on GD 20/21 (p=0.008), but not on GD 19. Conversely, female fetuses from DiBP-dosed dams had significantly higher AGD values than controls on both GDs 19 and 20/21 (p=0.005 and 0.032, respectively). Without using body weight as a covariate, AGD was significantly reduced in males at both time points (p=0.009) and significantly elevated in females at GD 20/21 (p=0.02) but not at GD 19 (p=0.057). At GD 20/21, ovarian estradiol levels were very low (near the detection limit of the assay), and no statistically significant differences between groups were observed (data not shown) (Boberg et al., 2008). At GD 20/21, testicular testosterone content and testicular testosterone production ex vivo were significantly reduced compared with controls (approximately 90% of controls, $p < 0.001$, respectively; data shown graphically) (Borch et al., 2006). Statistical comparison of data from the two age groups (GDs 20 and 21) showed no relevant differences. Testosterone levels were comparable among controls and treated rats at GD 19. As shown in Table B.12, at GD 20/21, histopathology revealed increased numbers of genocytes with increased localization in the seminiferous tubules due to vacuolization of Sertoli cell cytoplasm, and clustered Leydig cells with small cytoplasm and small irregular nuclei. At GD 19, only one or two of the examined testes demonstrated similar Sertoli cell and genocyte effects, but there was a significant increase in the clustering of small Leydig cells. Exposure to DiBP decreased plasma levels of insulin and leptin in fetuses at GD 21, indicating possible metabolic imbalances (Boberg et al., 2008). DiBP also decreased testicular mRNA levels for *insl-3* and several genes related to steroid synthesis, including scavenger receptor B-1 (SR-B1), steroidogenic acute regulated protein (StAR), P450 side chain cleavage (P450c17), and 17α-hydrolase/17,20-lyase (P450cc) at GDs 19 and 21 (Boberg et al., 2008). DiBP also decreased levels of PPARα mRNA in livers and testes of exposed males. Boberg et al. (2008) postulated that the down-regulation of receptor levels may be preceded by activation of PPARα or PPARγ by DiBP, which in turn may lead to the down-regulation of testosterone production, as PPARs regulate several genes involved in steroid synthesis.

The results indicate that 600 mg/kg-day DiBP administered on GDs 7–21 was a NOAEL for maternal toxicity in pregnant Wistar rats and a LOAEL in male fetuses for decreased AGD and testicular testosterone production and impaired testicular development indicated by clustering of small Leydig cells and Sertoli cell vacuolization (Borch et al., 2006). Other DiBP-induced changes in male fetuses included decreased plasma levels of insulin and leptin and decreased testicular mRNA levels for *insl-3*, several steroid synthesis genes, and PPARα (Boberg et al., 2008).

Table B.12. Testicular Histopathology in Male Rat Fetuses Exposed to DiBP from GDs 7–19 or 20/21				
	GD 19		GD 20/21	
Histology[a]	Control	600 mg/kg-day	Control	600 mg/kg-day
Clustering of small Leydig cells	2/13	9/9[b]	0/10	13/15[b]
Sertoli cells vacuolization	0/13	1/9	0/10	14/16[b]
Central localization of genocytes	0/13	2/9	0/10	14/16[b]
Multinuclear genocytes	1/13	0/9	1/10	10/16[c]

[a]One or two testes per litter were examined microscopically.
[b]Significantly different from controls, $p < 0.001$ (Fisher's exact test).
[c]Significantly different from controls, $p < 0.05$ (Fisher's exact test).

Source: Borch et al. (2006).

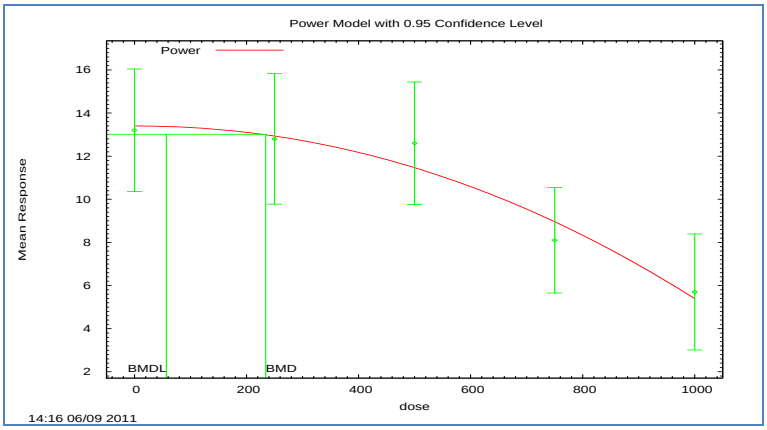
Swan et al. (2006, 2005) and Marsee et al. (2006)

Swan et al. (2006, 2005) and Marsee et al. (2006) identified a significant (p=0.007) association between prenatal maternal urinary levels of MIBP and reduced AGD and AGI (AGI = AGD/body weight) in human male infants. Boys whose mothers had the highest prenatal MIBP concentrations (75th percentile) had statistically significant ($p < 0.05$) shorter-than-expected AGIs compared to boys whose mothers had lower MIBP concentrations (25th percentile) (odds ratio = 9.1; confidence interval = 2.3–35.7) (Swan et al., 2006, 2005). In similar comparisons between 75th and 25th percentile values, significant ORs were found for mono-n-butyl phthalate, mono-benzyl phthalate, and mono-ethyl phthalate. However, Swan et al. (2005) noted several limitations to this study including: (1) only a single prenatal urine sample from each woman was obtained fairly late in pregnancy (mean=28.3 weeks), which may not reflect the most sensitive period of gestational development of the male reproductive tract; (2) the analyses were based on only a single measurement of AGD taken in boys of differing ages (the optimal timing for measurement of AGD in boys has not been established and the reliability of this measure as an index of impaired reproductive tract development in humans has not been firmly established); and (3) the study is based on a small number of subjects (134 boys and their mothers). Marsee et al. (2006) used measured maternal urinary levels of MIBP (and other dialkyl phthalate ester metabolites) and a simple pharmacokinetic model to estimate daily intakes of the mothers to parent dialkyl phthalate esters. The median DiBP intake was estimated to be 0.12 µg/kg-day with a 95th percentile dose of 0.41 µg/kg-day.

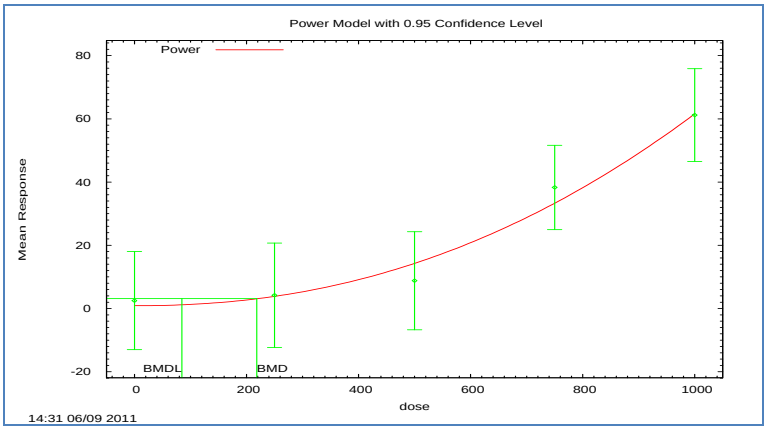
In a follow-up study, Swan et al. (2010) examined possible associations between play behavior scores for a group of boys (n=74) and girls (n=71) and concentrations of phthalate metabolites in samples of prenatal (mid-pregnancy) urine collected from their mothers participating in the Future Families Cohort Study. The relationship between play behavior scores and prenatal urinary phthalate metabolite concentrations was examined separately for boys and girls. Play behavior was assessed using completed Pre-School Activities Inventory questionnaires completed by the mothers and scored as masculine (male-typical play behavior), feminine (female-typical play behavior), or composite (total). Mean MIBP (recognized as a metabolite of dibutyl phthalate in this study) concentrations in prenatal urine from mothers of boys and girls were similar (4.0 ng/mL in mothers of boys and 4.1 ng/mL in mothers of girls). After adjusting for child's age, mother's age and education, and parental attitudes towards atypical play choices, MIBP concentrations in maternal urine were associated with a negative composite score (coefficient of -4.53, p=0.01) and a positive feminine score (coefficient of 2.48, p=0.07) in boys. The weak negative association (coefficient of -1.65) between MIBP urine concentrations and the masculine score in boys was unremarkable. There were no significant associations between maternal urinary MIBP concentrations and play behavior scores for girls.

Appendix C. BMD_{10} and $BMDL_{10}$ Summaries

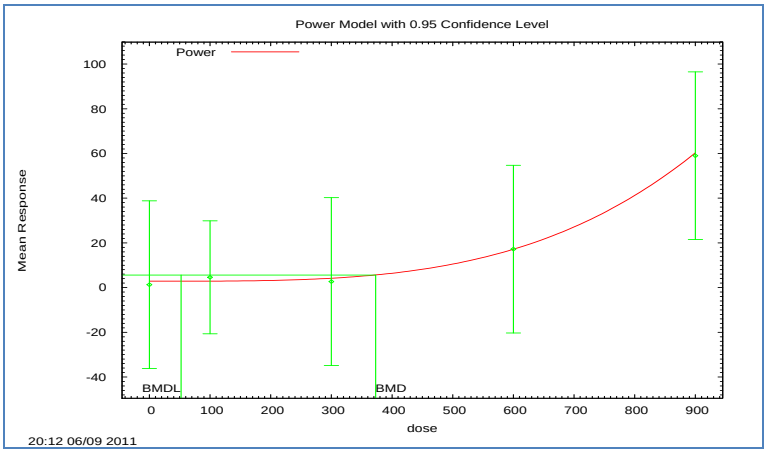
Live Fetuses per Litter (Gavage; Gd 6-20; Saillenfait et al., 2005)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	53	39	0.21	230.7
Polynomial	286	53	0.46	229.7
Power	234	57	0.45	229.8



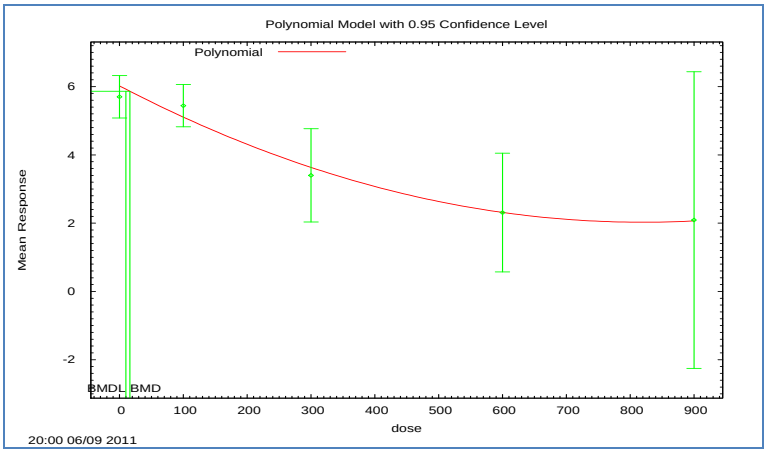
Percent Resorptions per Litter (Gavage; Gd 6-20; Saillenfait et al., 2005)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	39	30	0.05	430.9
Polynomial	291	62	0.53	426.2
Power	218	85	0.5	426.4



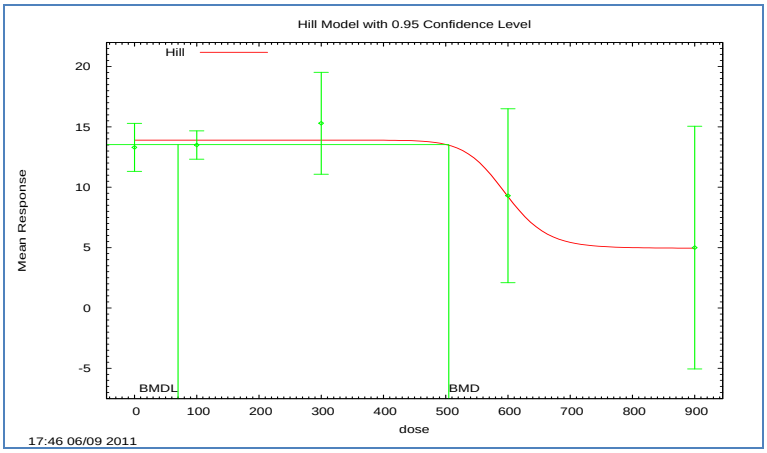
Fetal Mortality (Gavage; Gd 8-18; Howdeshell et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	50	33	0.41	222.3
Polynomial	437	45	0.89	221.6
Power	373	52	0.97	221.4



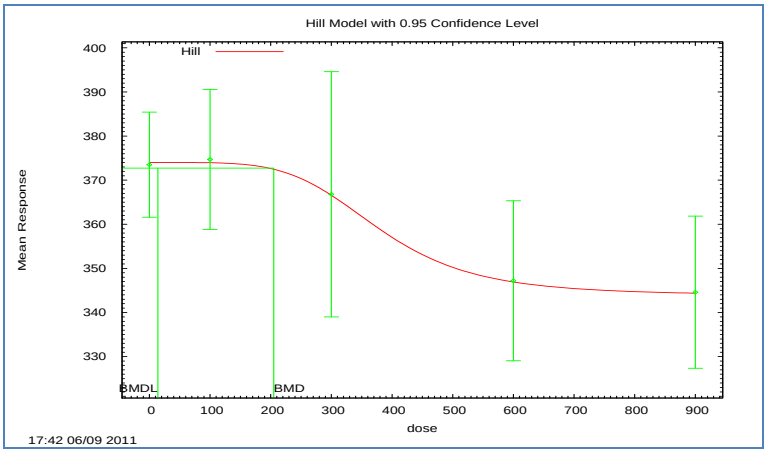
Fetal Testicular Testosterone Production (Gavage; Gd 8-18; Howdeshell et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	37	26	0.4	61.2
Polynomial	16	9.8	0.71	60.9
Power	37	26	0.4	61.2



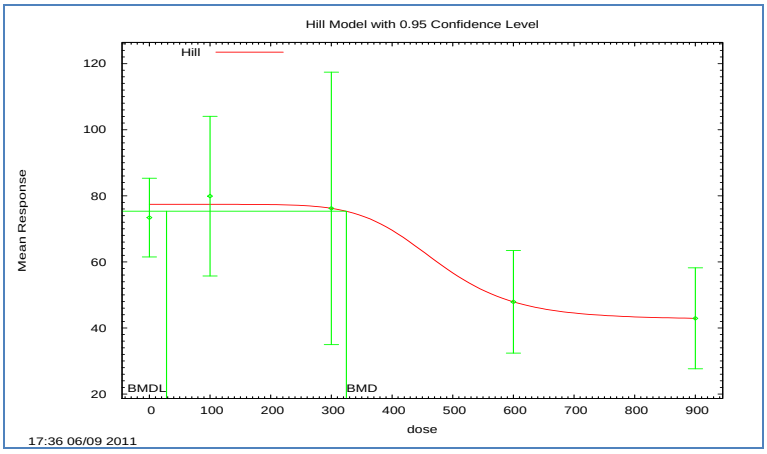
Number Live Fetuses (Gavage; Gd 8-18; Howdeshell et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Hill	505	70	0.68	115.6
Linear	45	30	0.24	117
Polynomial	401	43	0.48	116.3
Power	240	39	0.41	116.6



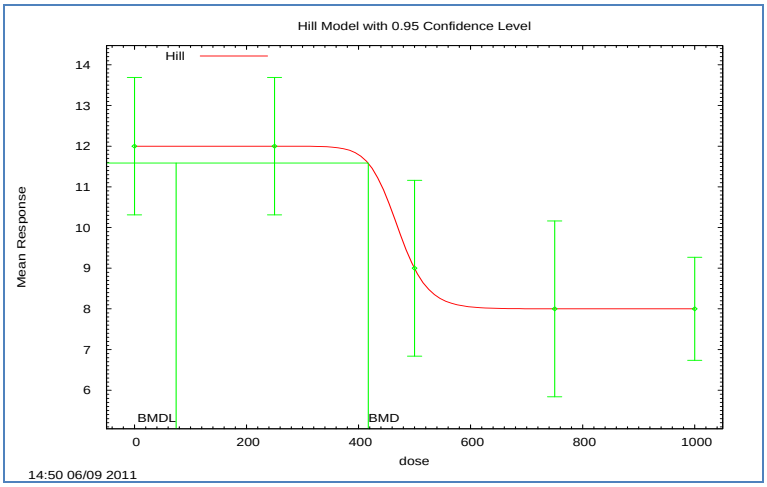
Maternal Body Weight Gd 18 (Gavage; Gd 8-18; Howdeshell et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Hill	205	14	0.89	190.7
Linear	41	28	0.72	188
Polynomial	32	14	0.53	190
Power	42	28	0.51	190



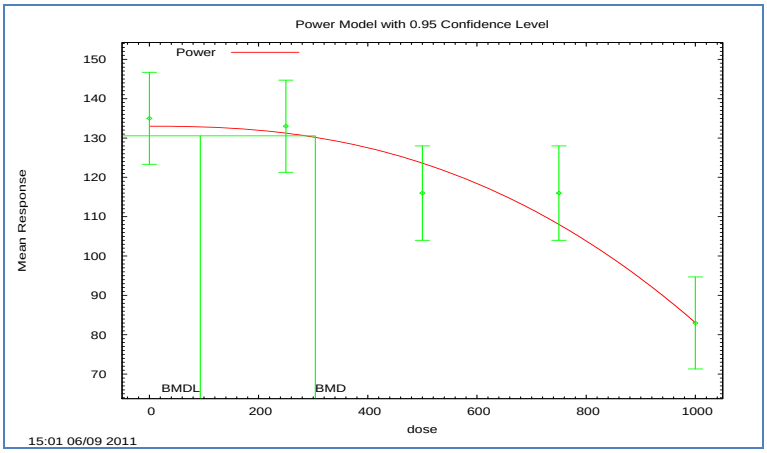
Maternal Body Weight Gain (Gavage; Gd 8-18; Howdeshell et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Hill	325	28	0.58	207.7
Linear	49	32	0.53	205.5
Polynomial	78	20	0.36	207.4
Power	107	33	0.4	207.2



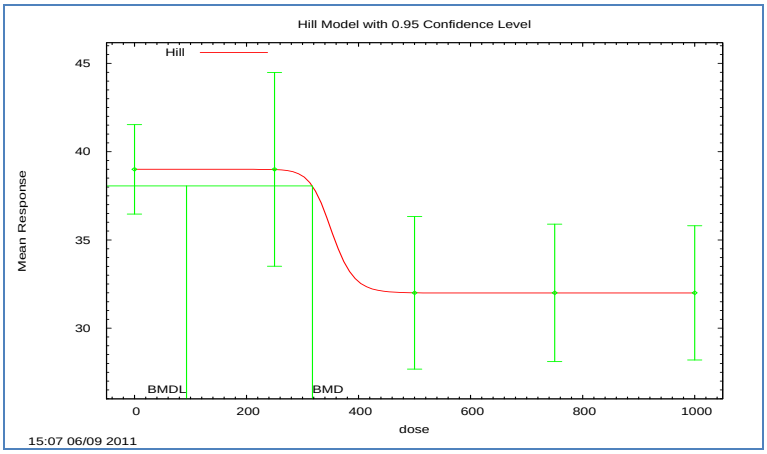
Maternal Body Weight Change Gd 6-9 (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Hill	417	74	1	462.5
Linear	88	63	0.36	463.7
Polynomial	61	32	0.24	465.3
Power	88	63	0.36	463.7



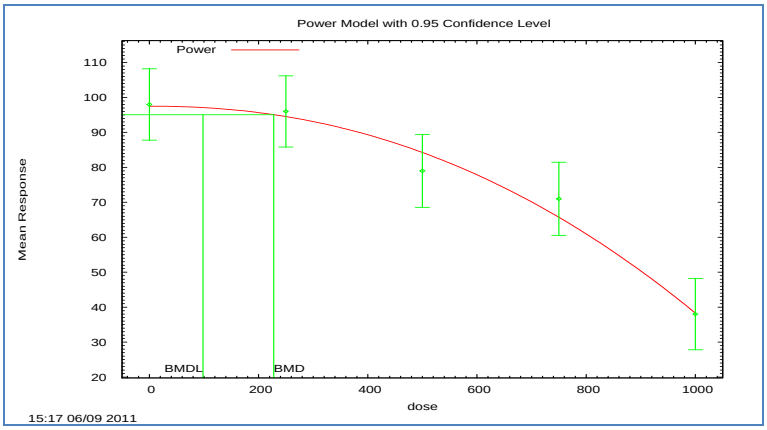
Maternal Body Weight Change Gd 6-21 (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Hill	305	94	0.05	910.7
Linear	58	46	0.04	911.3
Polynomial	264	70	0.14	908.8
Power	304	93	0.15	908.7



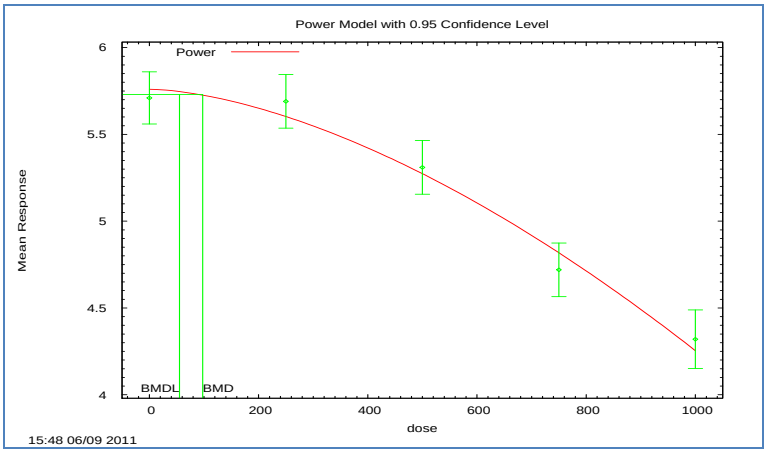
Maternal Body Weight Change Gd 15-18 (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Hill	318	93	1	656.3
Linear	115	76	0.29	658.1
Polynomial	61	32	0.23	659.2
Power	115	76	0.29	658.1



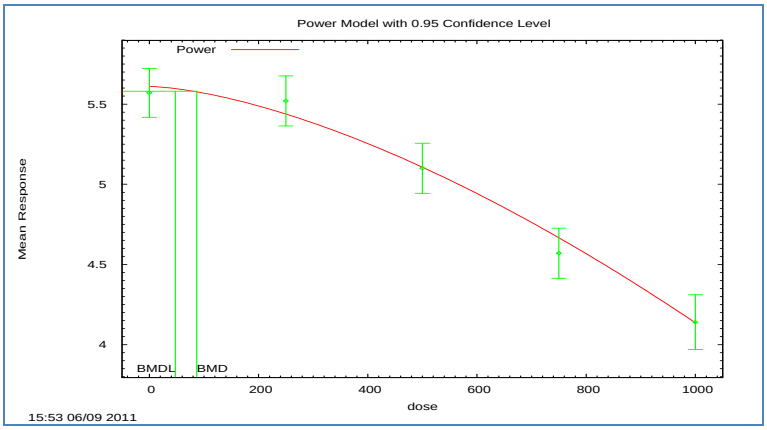
Maternal Gravid Uterus Weight (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Hill	229	99	0.13	876.8
Linear	42	35	0.015	880.9
Polynomial	222	68	0.31	874.8
Power	228	98	0.31	874.8



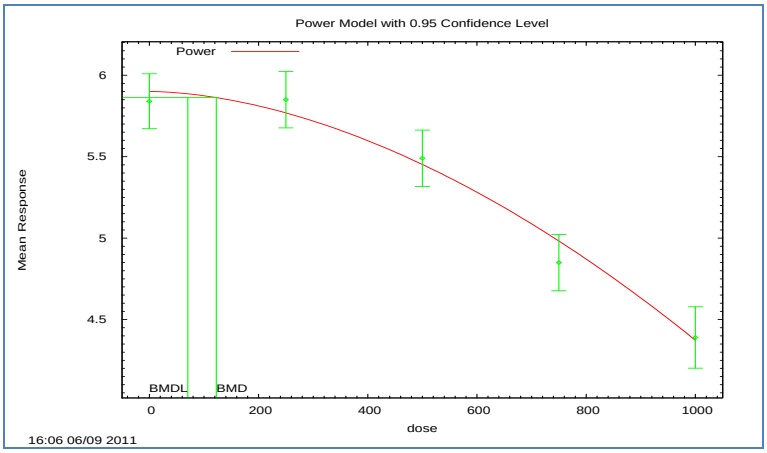
Fetal Body Weight, All Fetuses (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	24	21	0.0006	-101.3
Polynomial	75	36	0.06	-111
Power	98	55	0.1	-112.1



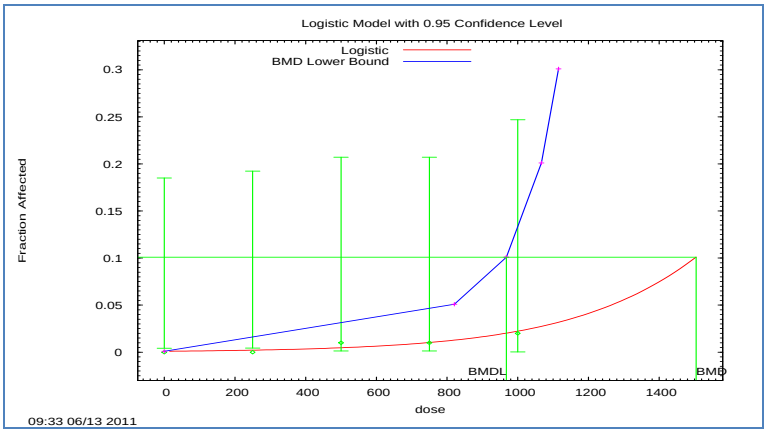
Fetal Body Weight, Female Fetuses (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	24	20	0.004	-103
Polynomial	60	32	0.13	-110.4
Power	87	48	0.23	-111.6



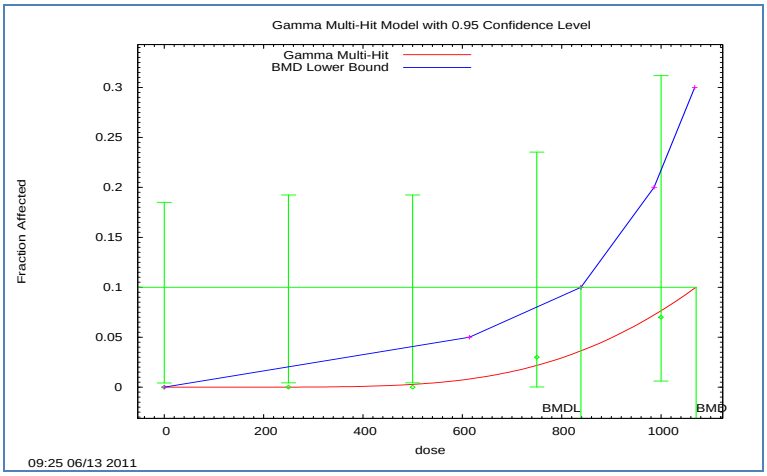
Fetal Body Weight, Male Fetuses (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	26	23	0.0002	-76.3
Polynomial	120	48	0.09	-88.9
Power	123	70	0.12	-89.4



Incidence of Cleft Sternum Skeletal Malformation per Litter (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	2531	971	0.99	12.4
Logistic	1505	968	0.98	12.5
Probit	1622	966	0.98	12.4
Weibull	2444	970	0.99	12.4

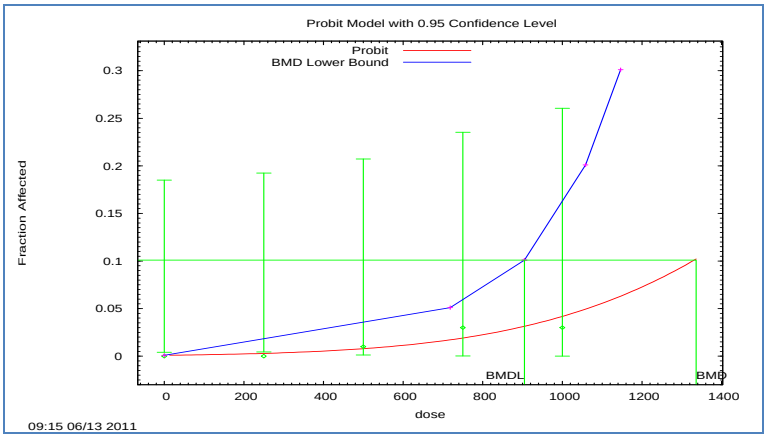


Incidence of Fused and Scrambled Sternebrae Skeletal Malformations per Litter (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	1071	839	0.99	19
Multistage	.	.	0.59	11.7
Logistic	1046	871	0.96	19.2
Probit	1054	856	0.98	19.1
Weibull	1065	847	0.98	19.1

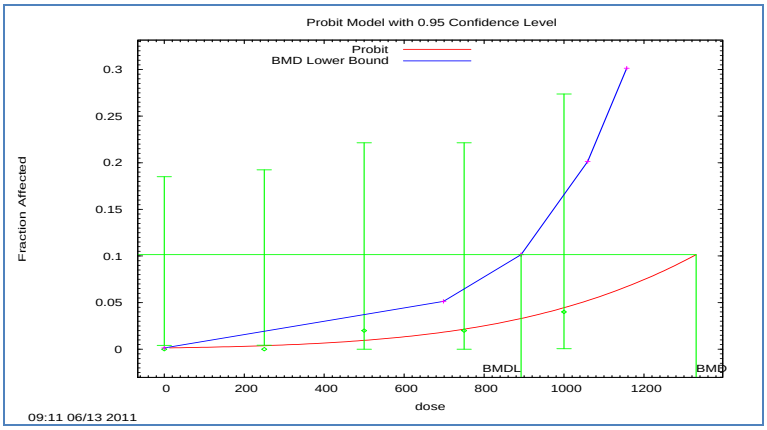


Incidence of Fused Sternebrae Skeletal Malformations per Litter (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	980	761	0.92	25
Multistage	2469	931	0.68	19.2
Logistic	984	812	0.84	25.4
Probit	978	792	0.88	25.2
Weibull	985	768	0.91	25.1

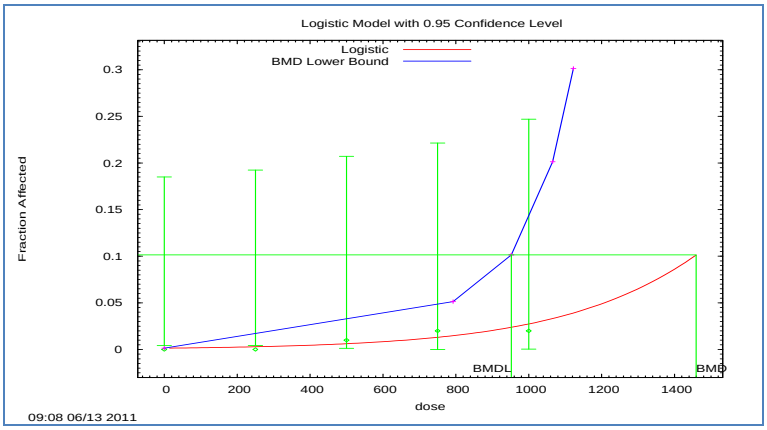
Incidence of Fused Thoracic or Lumbar Hemicentric Skeletal Malformations per Litter (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	1636	895	0.98	17.1
Logistic	1290	916	0.95	17.3
Probit	1336	905	0.96	17.2
Weibull	1611	901	0.98	17.1



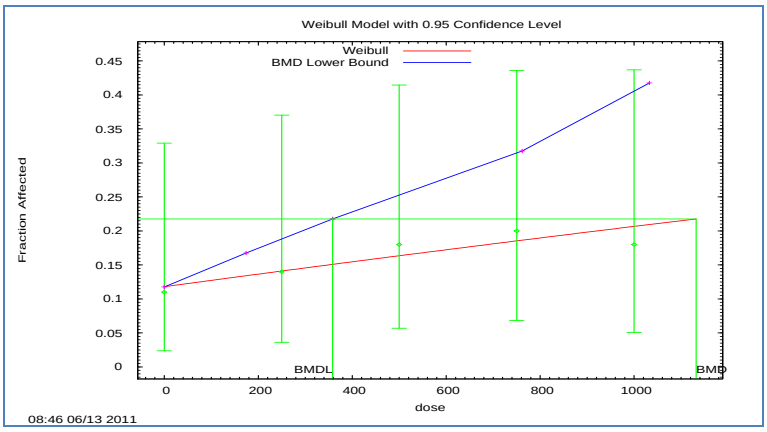
Incidence of Fused Thoracic or Lumbar Misaligned Skeletal Malformations per Litter (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	1689	881	0.98	18.5
Logistic	1279	905	0.94	18.7
Probit	1330	893	0.95	18.7
Weibull	1659	886	0.98	18.5



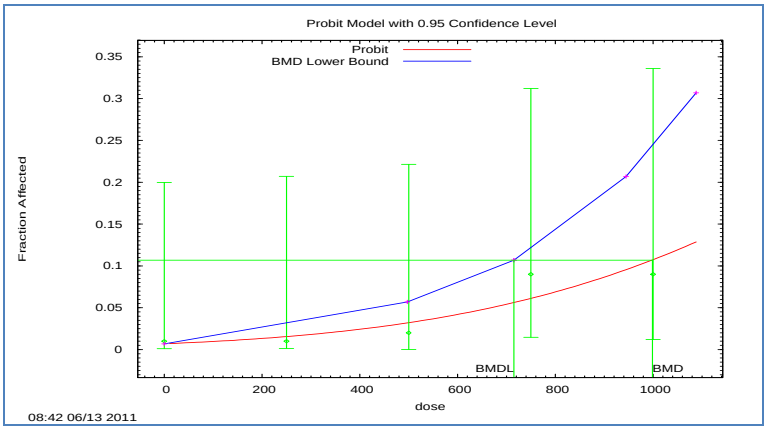
Incidence of Fused Thoracic or Lumbar Vertebral Arch Skeletal Malformations per Litter (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	2240	947	0.99	14.1
Logistic	1459	952	0.97	14.3
Probit	1543	946	0.97	14.3
Weibull	2195	949	0.99	14.1



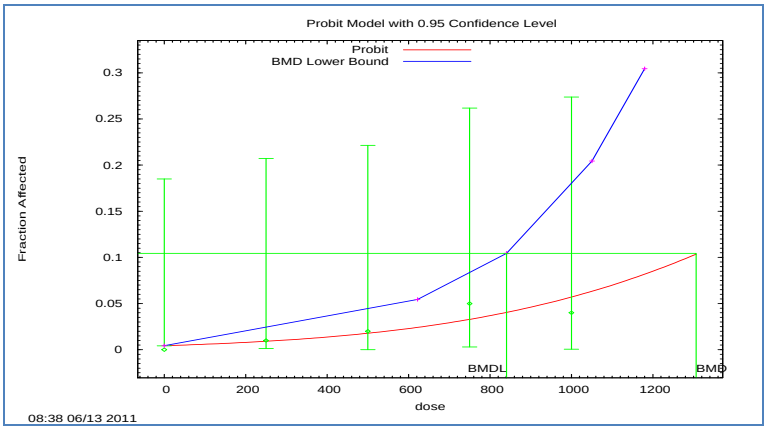
Incidence of Litters with 14th Any Supernumerary Ribs (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	1132	358	0.98	94.2
Multistage	1023	367	0.89	84
Logistic	1158	516	0.98	94.3
Probit	1153	493	0.98	94.2
Weibull	1132	358	0.98	94.2



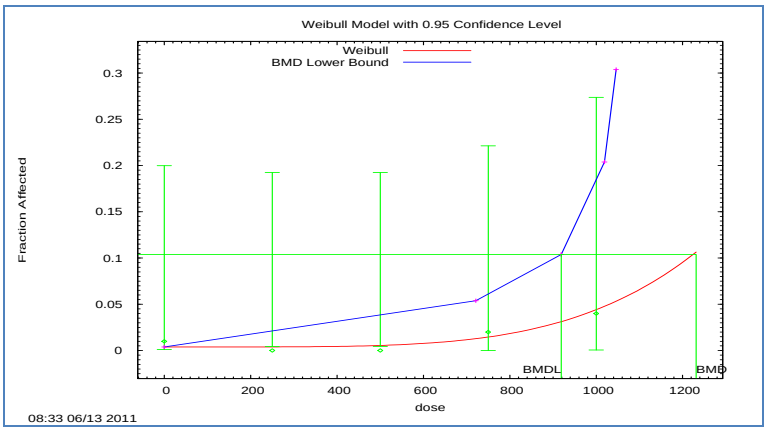
Incidence of Litters with 14th Long Supernumerary Ribs (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	995	652	0.81	38.9
Multistage	2430	916	.	19.1
Logistic	995	742	0.9	37.1
Probit	999	715	0.91	37
Weibull	997	653	0.8	39



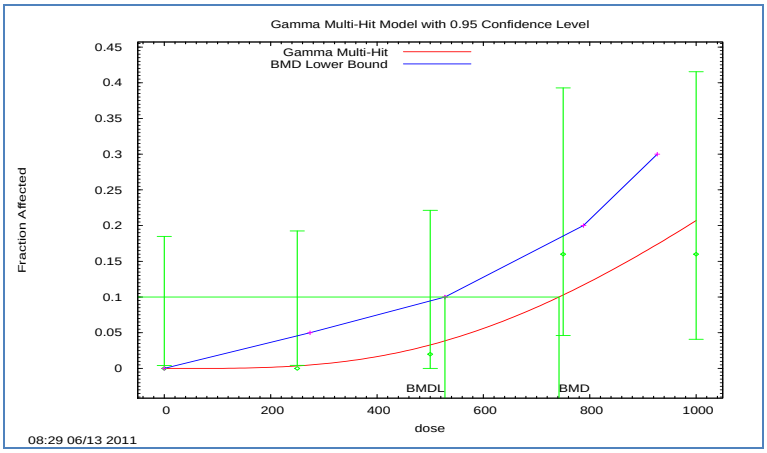
Incidence of Litters with Bipartite Sternebrae (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	1910	807	0.98	25
Multistage	.	.	0.7	12.3
Logistic	1277	859	0.93	25.4
Probit	1306	840	0.94	25.3
Weibull	1908	810	0.98	25



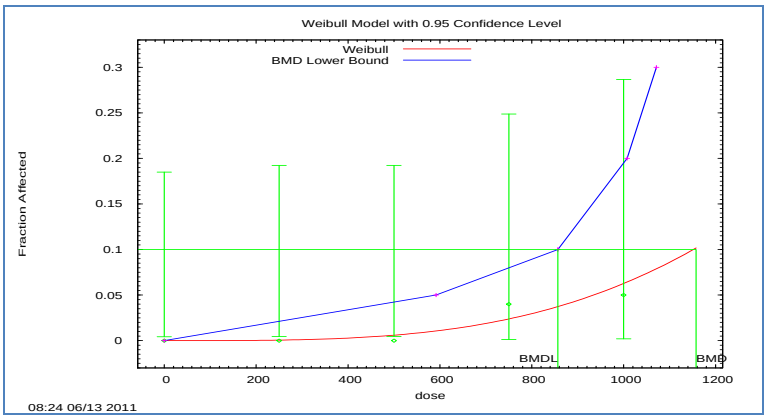
Incidence of Litters with Dilated Renal Pelves (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	1263	911	0.79	19.2
Logistic	1433	929	0.84	17.5
Probit	1607	933	0.84	17.6
Weibull	1231	919	0.79	19.2



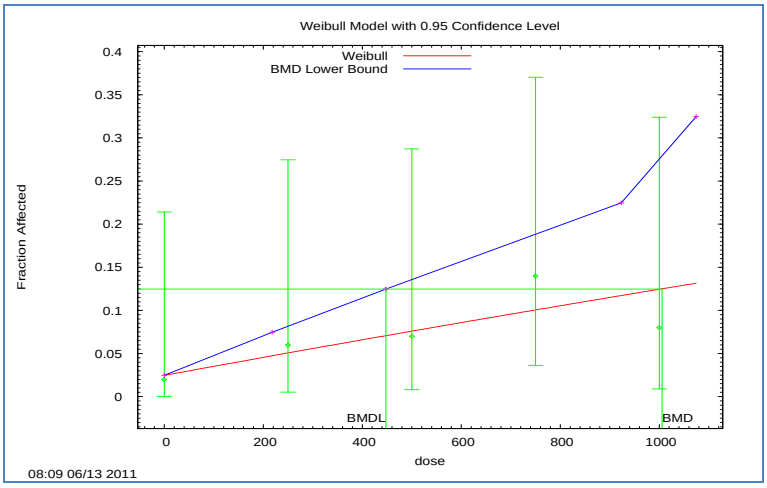
Incidence of Litters with Ectopic Testis Variations (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	742	528	0.76	43.6
Multistage	966	501	0.46	35.9
Logistic	798	648	0.54	44.7
Probit	776	621	0.62	44.2
Weibull	750	521	0.73	43.8



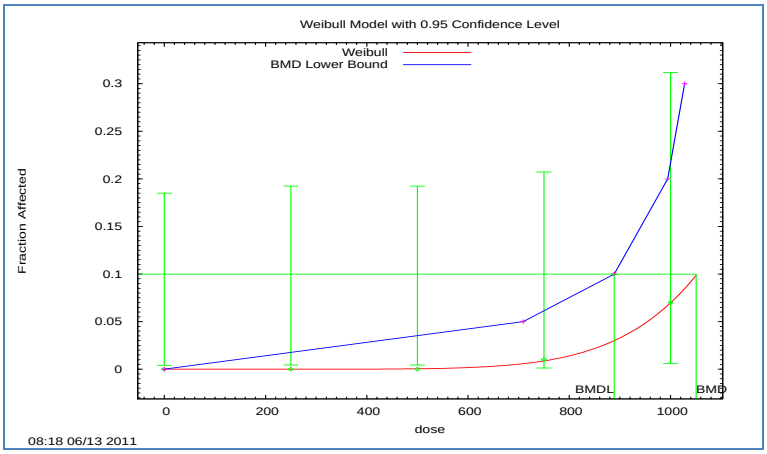
Incidence of Litters with Hydroureter Variations (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	1164	848	0.94	18.7
Logistic	1104	881	0.88	19
Probit	1117	866	0.9	18.8
Weibull	1158	857	0.94	18.7



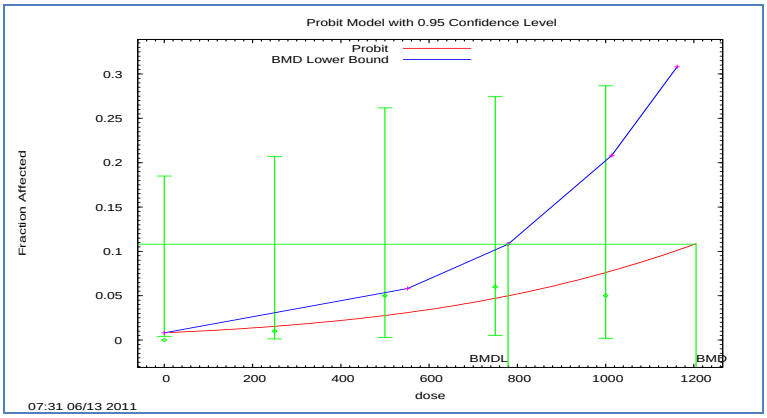
Incidence of Litters with Incomplete Ossification of Thoracic or Lumbar Vertebral Centra (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	1006	448	0.86	56.3
Logistic	1095	661	0.78	56.6
Probit	1079	630	0.79	56.6
Weibull	1006	448	0.86	56.3



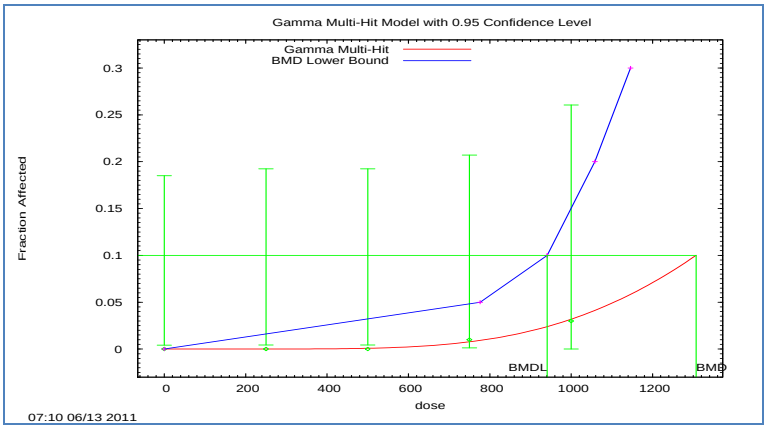
Incidence of Litters with Incomplete Ossification or Absent Hyoid Skeletal Variations (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	4400	756	0.09	21.4
Multistage	.	.	0.65	11.7
Logistic	1041	903	1	15.5
Probit	1051	891	1	15.5
Weibull	1051	889	1	15.5



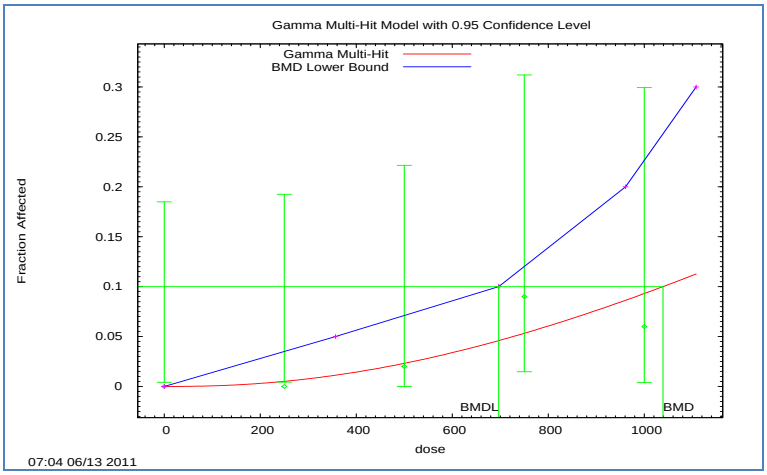
Incidence of Litters with Incompletely Ossified Sternebrae (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	1487	685	0.99	29.7
Multistage	1205	805	0.82	32.5
Logistic	2496	941	0.83	20.6
Probit	1205	779	0.83	32.4
Weibull	1487	685	0.99	29.7



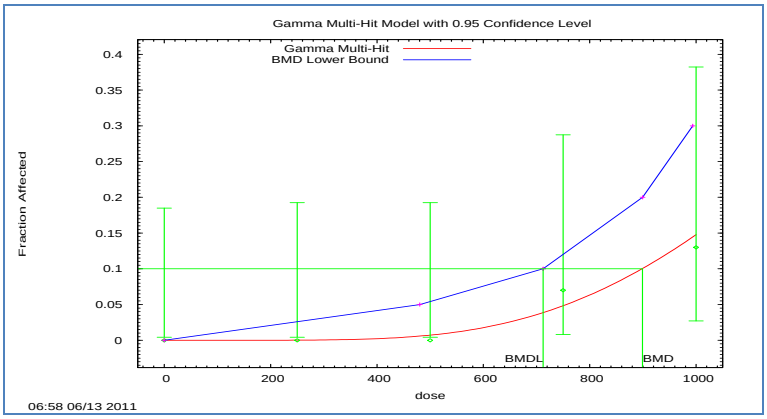
Incidence of Litters with Kidney and Ureter Absent, Unilateral or Bilateral Malformation (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	1307	941	1	11.3
Logistic	1185	953	1	11.3
Probit	1231	946	1	11.3
Weibull	1265	947	1	11.3



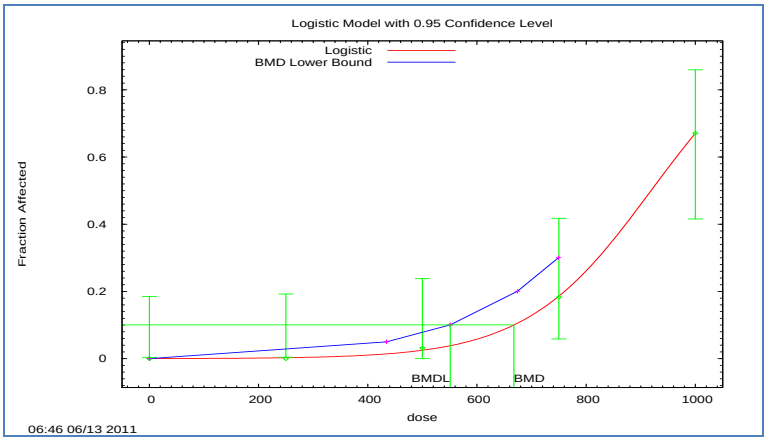
Incidence of Litters with Rudimentary Cervical Ribs (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	1039	697	0.82	30
Multistage	2495	941	0.59	19.2
Logistic	1026	786	0.67	30.6
Probit	1014	760	0.71	30.4
Weibull	1047	699	0.81	30



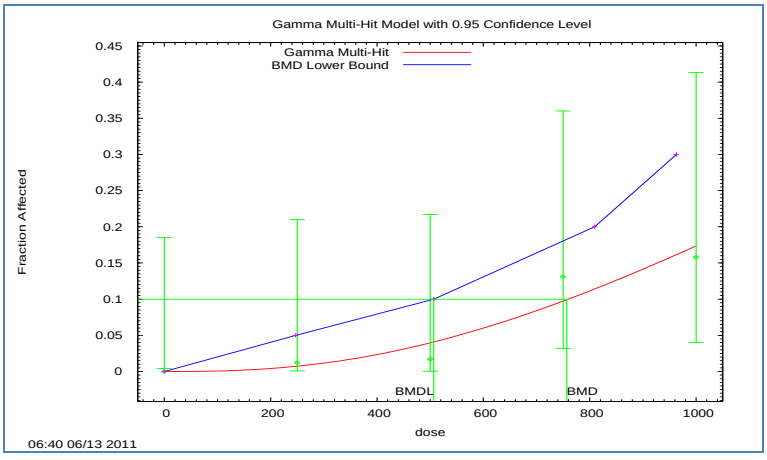
Incidence of Total Sternebra Skeletal Malformation per Litter (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	899	713	0.94	29.1
Multistage	1690	744	0.59	25.2
Logistic	921	770	0.85	29.6
Probit	909	748	0.89	29.3
Weibull	908	717	0.92	29.2



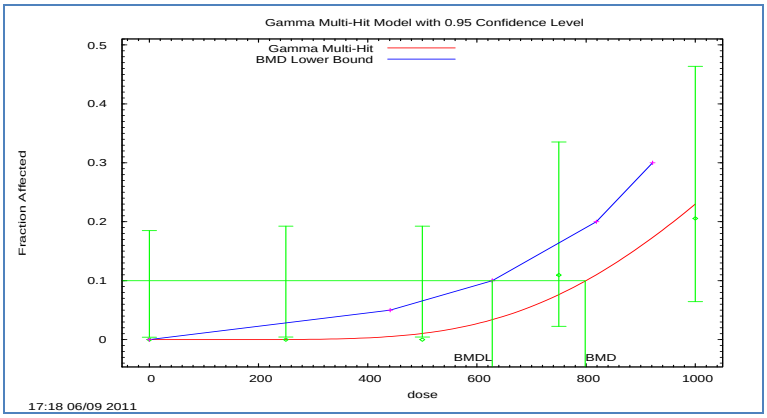
Mean Percent Fetuses with Skeletal Malformations per Litter (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	642	528	0.89	53.3
Multistage	284	191	0.003	63.6
Logistic	667	551	0.99	52.9
Probit	653	536	0.97	53
Weibull	655	532	0.99	52.8



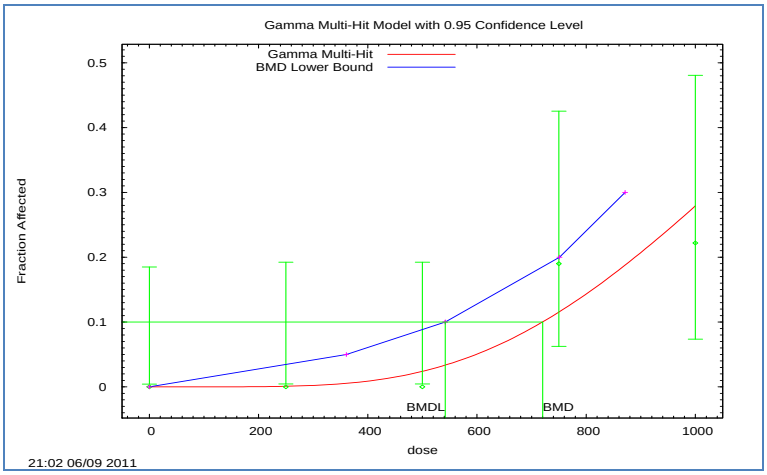
Mean Percent Fetuses with Visceral Malformations per Litter (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	757	507	0.89	43.1
Multistage	1237	600	0.47	30.9
Logistic	819	661	0.78	43.5
Probit	798	632	0.83	43.2
Weibull	764	506	0.88	43.1



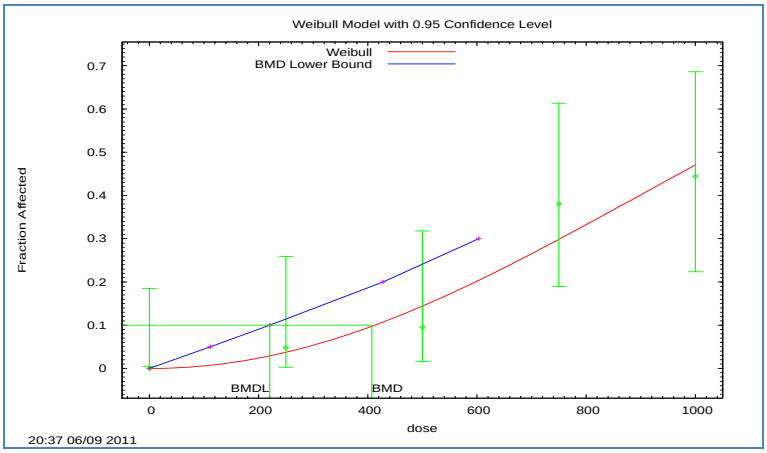
Mean Percent Fetuses with External Malformations per Litter (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	798	628	0.89	37.6
Multistage	979	508	0.39	35.4
Logistic	830	691	0.75	38.3
Probit	815	669	0.82	37.9
Weibull	808	628	0.86	37.8



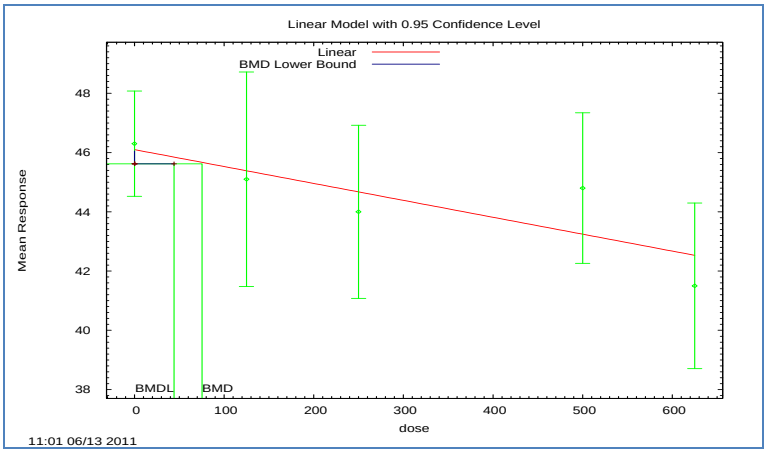
Percent of Litters with External Malformations (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	720	542	0.58	45.8
Multistage	804	440	0.19	40.1
Logistic	759	621	0.36	47.1
Probit	742	599	0.45	46.5
Weibull	725	533	0.53	46.2



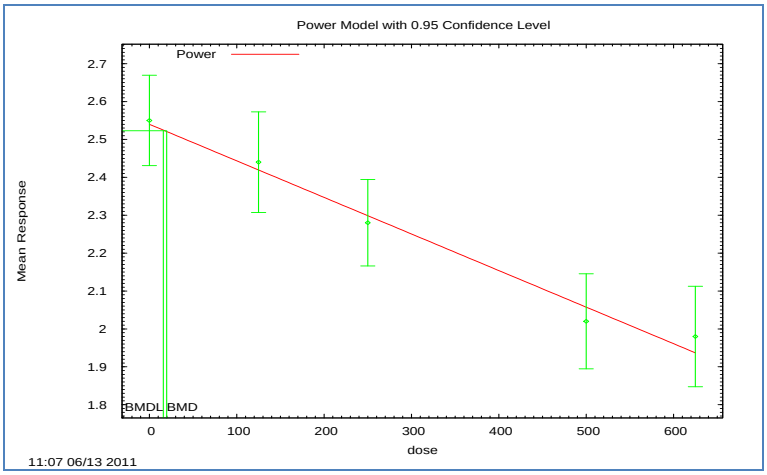
Percent of Litters with Visceral Malformations (Gavage; Gd 6-20; Saillenfait et al., 2006)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	405	221	0.76	79.1
Multistage	252	173	0.41	74.6
Logistic	495	393	0.56	80.3
Probit	470	368	0.65	79.7
Weibull	407	220	0.76	79.1



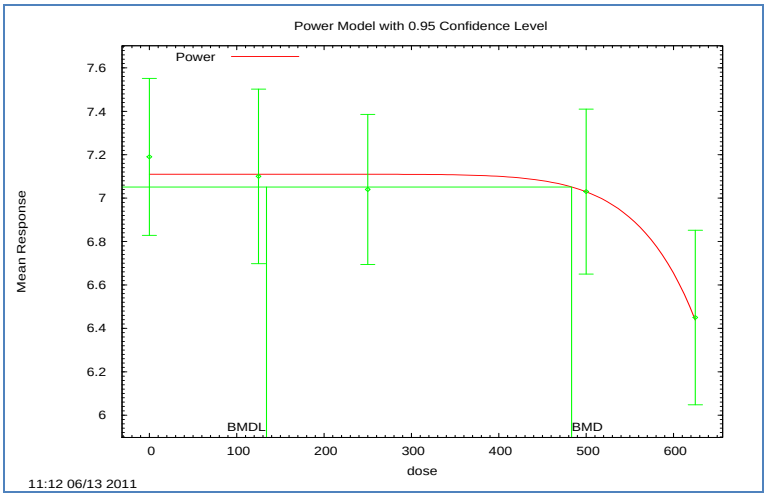
Female Pup BW PND 21 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	75	44	0.44	241.3
Polynomial	115	23	0.27	243.2
Power	75	44	0.44	241.3



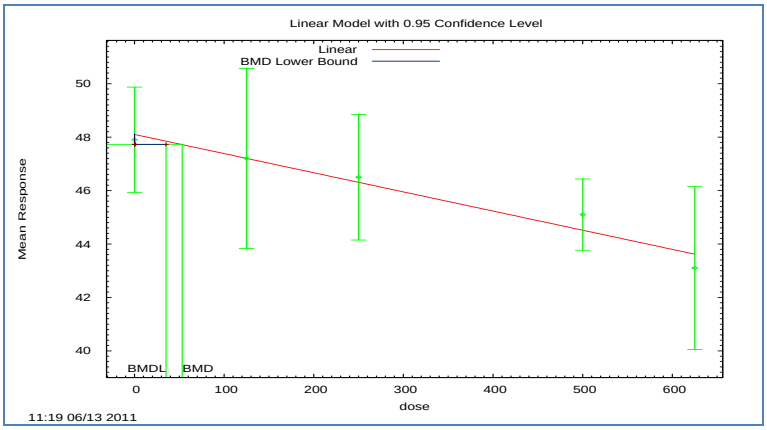
Male Pup Anogenital Distance PND 1 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	19.8	16	0.72	-134.7
Polynomial	15	9.7	0.69	-133.4
Power	19.8	16	0.72	-134.8



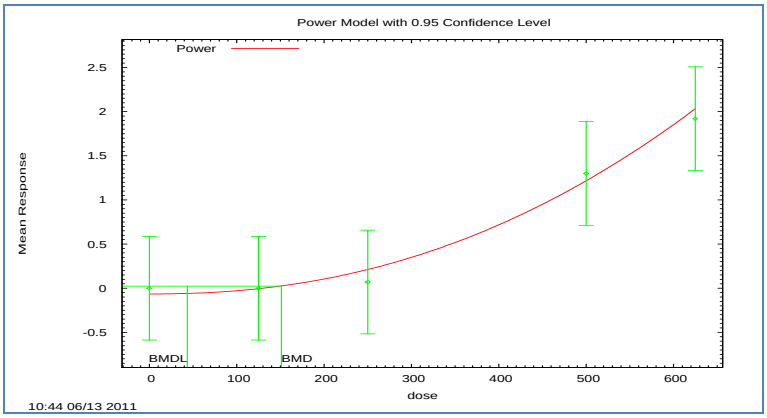
Male Pup Body Weight PND 1 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	65.5	40.4	0.28	8
Polynomial	316	38	0.29	8
Power	483	134	0.79	8



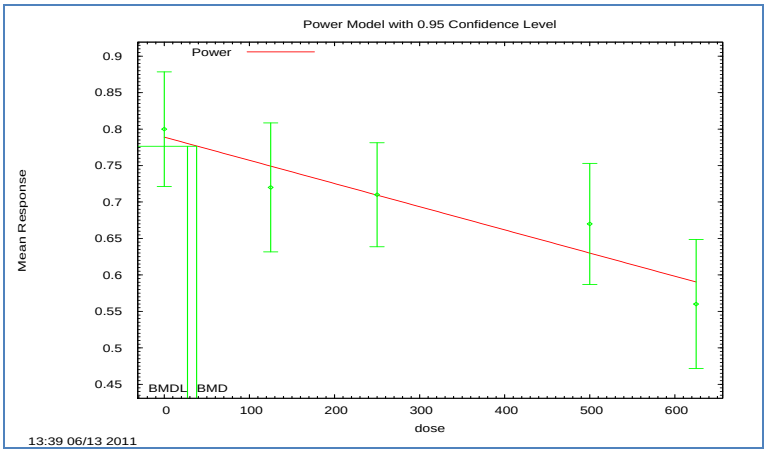
Male Pup Body Weight PND 21 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	53	35	0.9	226.8
Polynomial	115	23	0.88	228.5
Power	142	36	0.86	228.6



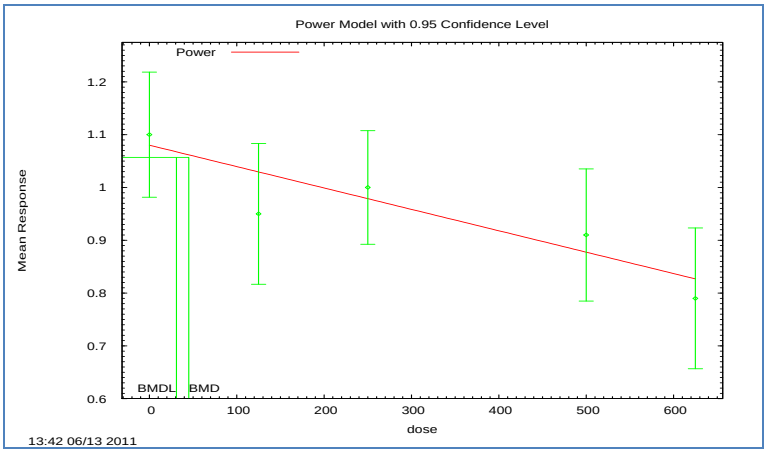
Number of Areolas/Nipples per Mature Rat (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	28	22	0.18	56.2
Polynomial	204	36	0.79	53.8
Power	151	43	0.74	54



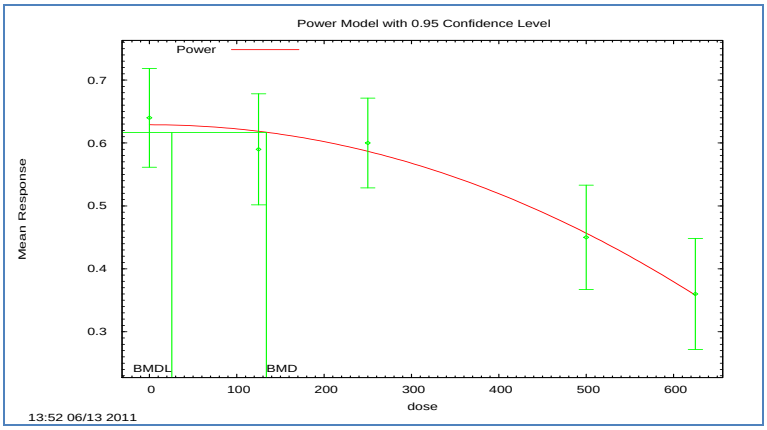
Pup Absolute Prostate PNW 11-12 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	38	27	0.46	-177.9
Polynomial	49	18	0.29	-176
Power	38	27	0.46	-177.9



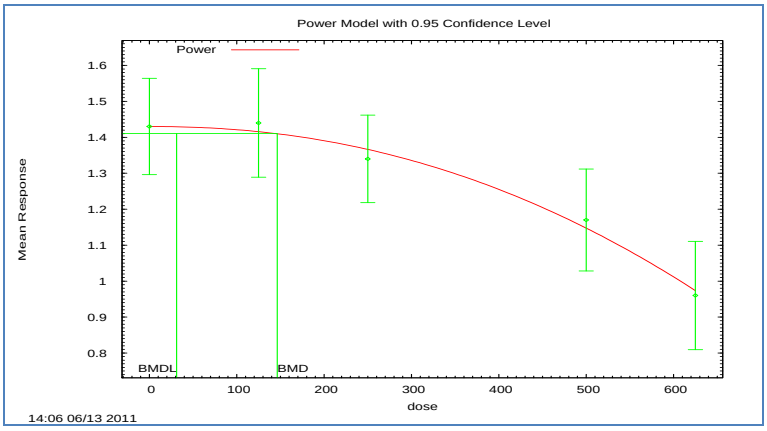
Pup Absolute Prostate PNW 16-17 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	45	31	0.39	-130.7
Polynomial	58	19	0.23	-128.8
Power	45	31	0.39	-130.7



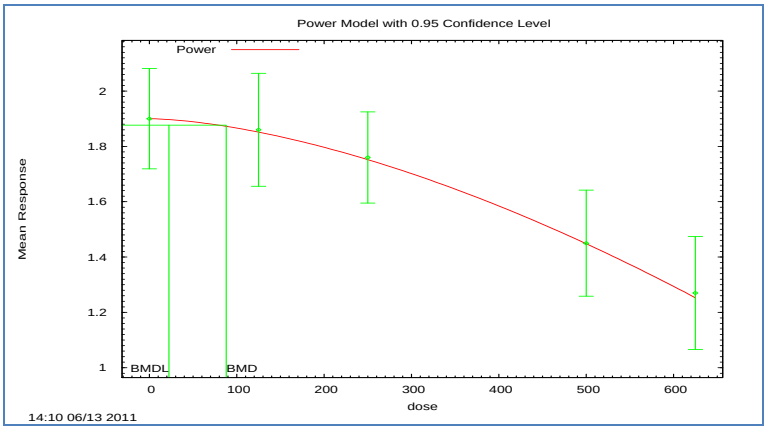
Pup Absolute Right Epididymis PNW 16-17 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	28	21	0.33	-177.3
Polynomial	117	26	0.64	-177.9
Power	134	26	0.64	-177.8



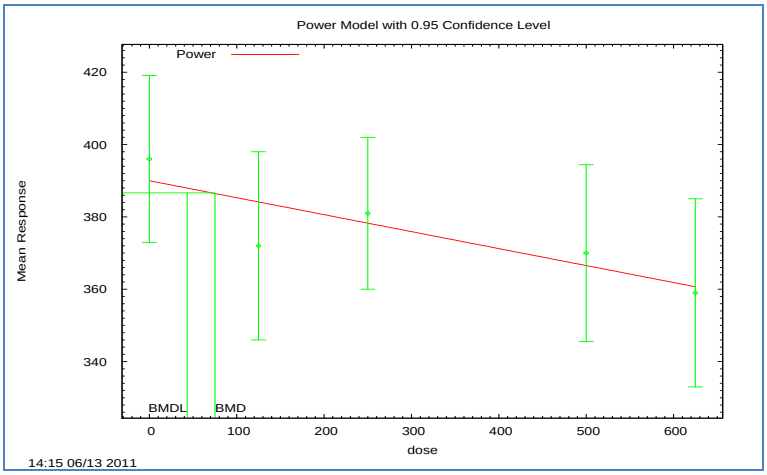
Pup Absolute Seminal Vesicle PNW 11-12 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	28	21	0.31	-116.1
Polynomial	144	28	0.75	-117.1
Power	146	31	0.75	-117.2



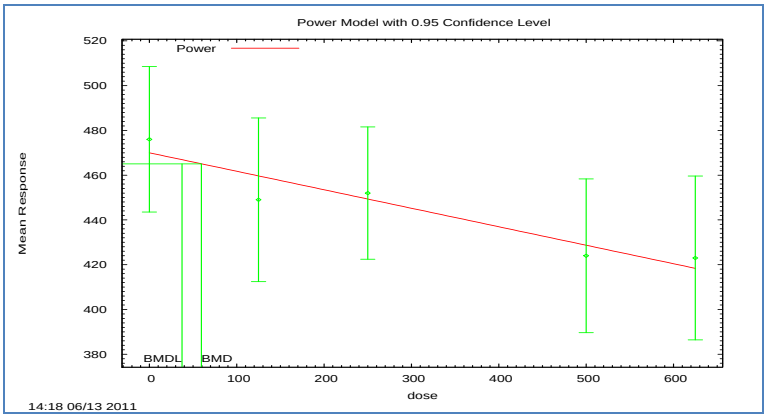
Pup Absolute Seminal Vesicle PNW 16-17 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	27	21	0.66	-83.5
Polynomial	71	21	0.97	-83.1
Power	88	22	0.99	-83.1



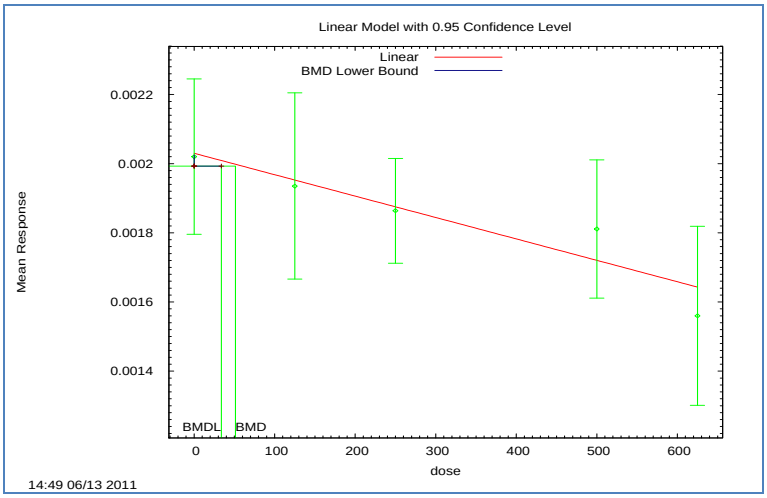
Pup Body Weight PNW 11-12 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	75	43	0.62	469.2
Polynomial	58	19	0.42	471.2
Power	75	43	0.62	469.2



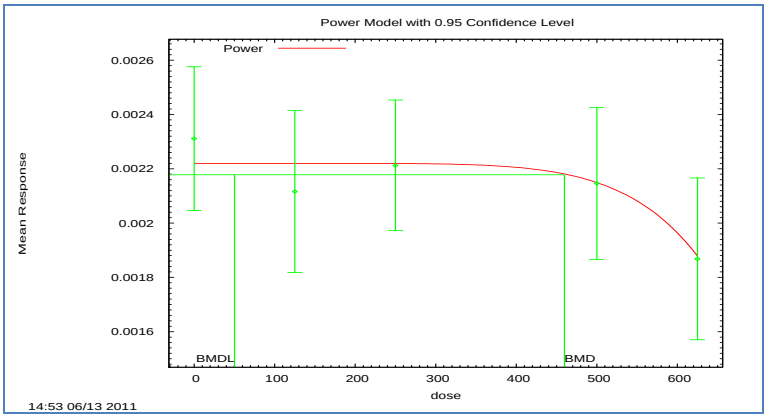
Pup Body Weight PNW 16-17 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	59.6	37.3	0.83	507
Polynomial	39	16	0.71	509
Power	60	37	0.83	507.2



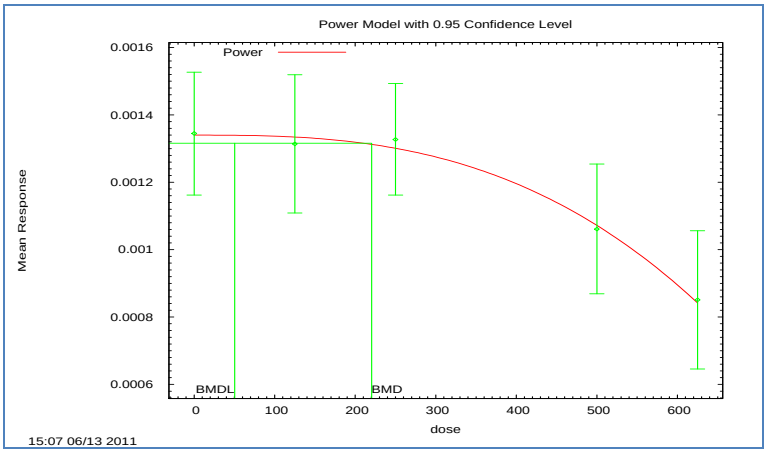
Pup Relative Prostate PNW 11-12 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	51	34	0.65	-855
Polynomial	102	23	0.51	-853.3
Power	139	34	0.48	-853.2



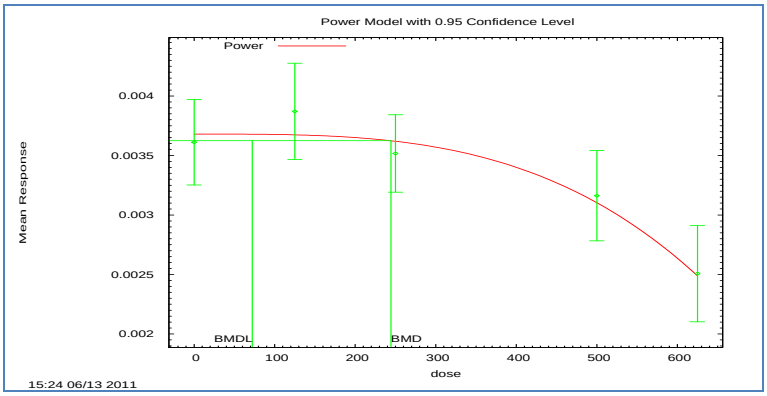
Pup Relative Prostate PNW 16-17 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	79	45	0.47	-827
Polynomial	231	28	0.34	-825.4
Power	460	50.2	0.52	-826.2



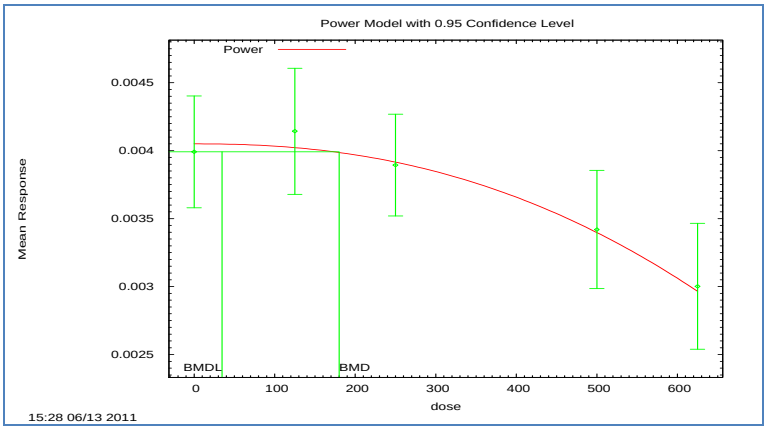
Pup Relative Right Epididymis PNW 16-17 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	37	26	0.29	-868.3
Polynomial	259	41	0.88	-869.8
Power	220	50	0.9	-869.8



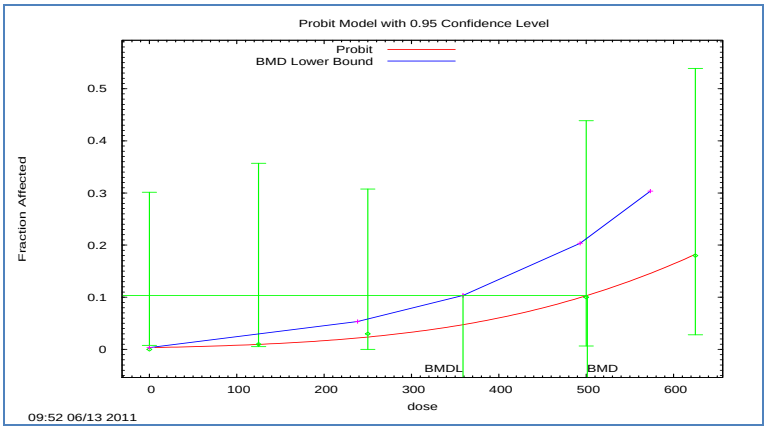
Pup Relative Seminiferous Vesicle PNW 11-12 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	33	24	0.05	-787.2
Polynomial	290	57	0.36	-790.9
Power	244	72	0.35	-790.8



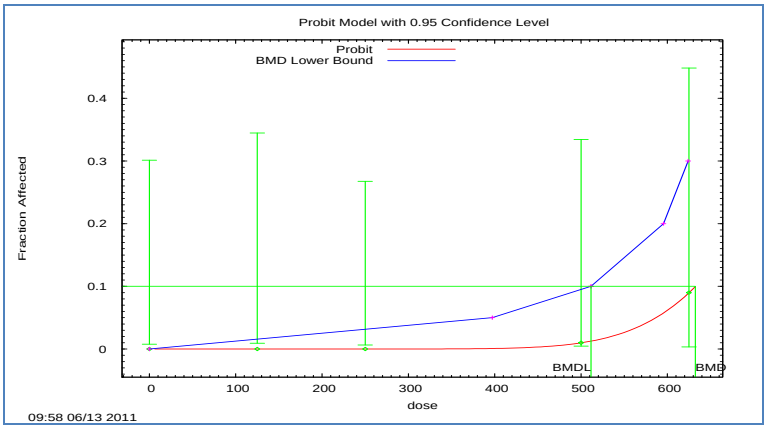
Pup Relative Seminiferous Vesicle PNW 16-17 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Linear	38	27	0.39	-776.2
Polynomial	231	35	0.82	-776.8
Power	180	35	0.78	-776.7



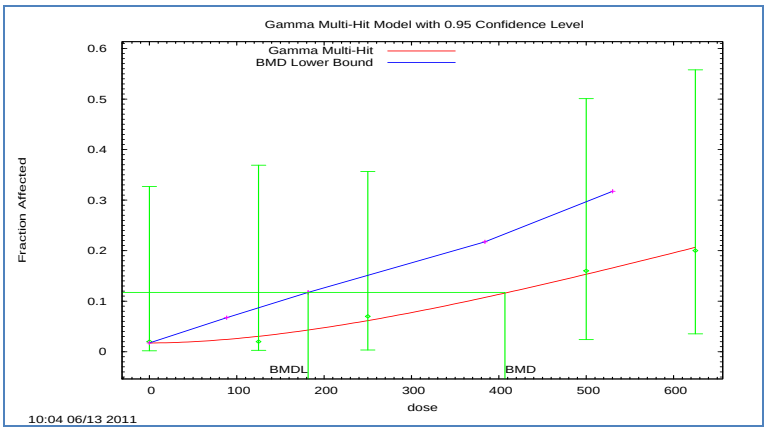
Incidence of Azoospermia in Epididymides PNW 11-12 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	465	236	1	25.5
Multistage	806	304	0.79	16.5
Logistic	515	380	0.99	25.6
Probit	501	359	1	25.6
Weibull	469	237	1	25.5



Incidence of Testiculr Interstitial Cell Hyperplasia PNW 11-12 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	2482	365	0.16	16.1
Logistic	631	523	1	11.3
Probit	632	512	1	11.3
Weibull	632	498	1	11.3



Incidence of Testicular Tubular Degeneration/Atrophy/Hypoplasia PNW 11-12 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	407	182	0.97	37.2
Multistage	539	237	.	21.1
Logistic	452	320	0.98	35.3
Probit	435	299	0.99	35.2
Weibull	410	181	0.96	37.2



Incidence of Testicular Tubular Necrosis PNW 11-12 (Gavage; Gd 12-21; Saillenfait et al., 2008)				
Model	**BMD_{10}**	**$BMDL_{10}$**	**P-value**	**AIC**
Gamma Multi-hit	749	474	0.87	14.4
Logistic	747	518	0.96	12.4
Probit	787	505	0.96	12.4
Weibull	739	476	0.87	14.4