UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
Bethesda, MD 20814

**Memorandum**

Date: October 24, 2010

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SUBJECT : Toxicity Review of **Dicyclohexyl phthalate (DCHP)**

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 **DCHP**.

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.

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**FINAL**

# TOXICITY REVIEW FOR DICYCLOHEXYL PHTHALATE (DCHP, CASRN 84-61-7)

Contract No. CPSC-D-06-0006
Task Order 012

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| | |
|---|---|
| **AGD** | Anogenital distance |
| **ALT** | Alanine aminotransferase |
| **AST** | Aspartate aminotransferase |
| **CEases** | Cholesterol esterases |
| **DBP** | Dibutyl phthalate |
| **DCHP** | Dicyclohexyl phthalate |
| **DEHP** | Di-2-ethylhexyl phthalate |
| **DEP** | Diethyl phthalate |
| **DHP** | Di-n-hexyl phthalate |
| **DMP** | Dimethyl phthalate |
| **DPeP** | Di-n-pentyl phthalate |
| **DPrP** | Di-n-propyl phthalate |
| **FSH** | Follicle-stimulating hormone |
| **GD** | Gestation day |
| **LH** | Luteinizing hormone |
| **LOAEL** | Lowest-observed-adverse-effect level |
| **MCHP** | Monocyclohexyl phthalate |
| **NOAEL** | No-observed-adverse-effect level |
| **PA** | Phthalic acid |
| **PND** | Postnatal day |
| **SD** | Standard deviation |
| **SEM** | Standard error of the mean |
| **TTM** | Trans-abdominal testicular migration |

## EXECUTIVE SUMMARY

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

Exposure to DCHP resulted in oral $LD_{50}$s >3200 mg/kg in four animal studies. Slight dermal irritation was noted in one well-described guinea pig study and mild dermal irritation was reported in a rabbit study. Slight eye irritation was reported in a rabbit study. DCHP was also reported to not be a sensitizer in guinea pigs by one information source. Insufficient data were available to make the determination of whether DCHP was associated with acute dermal or inhalation toxicity.

Sufficient evidence supported the conclusion that DCHP was a subchronic toxicant. Exposure to DCHP induced changes in body weight, and liver, kidney, and thyroid weight and pathology following subchronic administration. Sufficient animal data also existed to support the conclusion that DCHP was a reproductive and developmental toxicant. DCHP-induced reproductive effects and developmental effects were reported in both male and female reproductive systems and tissues (mean estrous cycle, homogenization resistant sperm, anogenital distance, areola without nipples, resorptions, live fetuses, number of pups born, reproductive organ weights, and incidence of hypospadias). DCHP-induced developmental effects also occurred in non reproductive tissues (incidence of cervical ribs). There was inadequate evidence to support the conclusion that DCHP was a neurotoxicant.

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.

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

When considering FHSA criteria, products that contain DCHP may be considered "hazardous" if short-term, or long-term exposures to the general population during "reasonably foreseeable handling and use" exceed the short-term or long-term ADI's for the general population (0.1 and 0.051 mg DCHP/kg bw-day, respectively).

In addition, products that contain DCHP may be considered “hazardous” if long-term exposures during “reasonably foreseeable handling and use” exceed the long-term ADI for reproductive effects (0.41 DCHP/kg bw-day, respectively).

In addition, products that contain DCHP 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 (0.68 mg DCHP/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 DICYCLOHEXYL PHTHALATE (DCHP, CASRN 84-61-7)

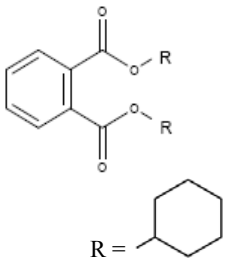
## 1. INTRODUCTION

This report provides the available data for the identity, physicochemical properties, manufacture and use, toxicity, and exposure information on dicyclohexyl phthalate (DCHP). Historically, concerns over the use of phthalates have been associated particularly with the potential for reproductive/developmental effects from a human health view point (NICNAS, 2008). In addition, concerns that the structural and physicochemical properties of certain phthalates used as plasticizers may permit migration and leaching resulting in potential human exposure, particularly in soft plastics (NICNAS, 2008). Combining the potential for exposure and a recognized toxicity profile for some particular phthalates has raised concerns over potential health risks, especially when used in consumer products (NICNAS, 2008).

## 2. IDENTITY AND PHYSICOCHEMICAL CHARACTERISTICS

This section highlights the identity and key physicochemical properties of DCHP. DCHP is considered to belong to the Transitional Phthalate Esters group, which are produced from alcohols with straight-chain carbon backbones of C4-6 (NICNAS, 2008). Structurally, DCHP has 2 branched ester side chains each with a side chain ring structure. The identity and physicochemical properties of DCHP can be seen in Tables 2.1 and 2.2 (NICNAS, 2008; HSDB, 2009).

**Table 2.1. Names, Structural Descriptors, and Molecular Formulas of DCHP (NICNAS, 2008)**

| | |
|---|---|
| CAS Number: | 84-61-7 |
| Chemical Name: | 1, 2-Benzenedicarboxylic acid, dicyclohexyl ester |
| Common Name: | Dicyclohexyl phthalate (DCHP) |
| Molecular Formula: | C20H26O4 |
| Structural Formula: |   |
| Molecular Weight: | 330.46 |
| Synonyms: | 1,2-Benzenedicarboxylic acid, dicyclohexyl ester; Phthalic acid, dicyclohexyl ester; Diclohexyl 1,2-benzenedicarboxylate; Dicyclohexyl phthalate |
| Purity/Impurities/Additives: | Impurity; phthalic acid (0.15%), water (0.1%) |

| Table 2.2. Physicochemical Properties of DCHP | |
|---|---|
| **Property** | **Value** |
| Physical state | White, crystalline solid (NICNAS, 2008); Mildly aromatic odor (HSDB, 2009) |
| Melting point | 66°C (NICNAS, 2008; HSDB, 2009) |
| Boiling point | 222-228°C (0.5 kPa) (NICNAS, 2008); 224°C (4mm Hg)(HSDB, 2009) |
| Density | 1383 kg/$m^3$ (20°C) (NICNAS, 2008; HSDB, 2009) |
| Vapor pressure | 13.3 x $10^{-3}$ kPa (150°C) (NICNAS, 2008) |
| Water solubility | 4 x $10^{-3}$ g/L (24°C) (NICNAS, 2008; HSDB, 2009) |
| Partition coefficient n-octanol/water (log Kow) | 3-4 (temp not specified) (NICNAS, 2008) 6.20 (est) (HSDB, 2009) |
| Henry's law constant | 1.0 x $10^{-7}$ atm-cu m/mol (25ºC) (HSDB, 2009) |
| Flash point | 207°C (NICNAS, 2008) |

## 3. MANUFACTURE, SUPPLY, AND USE

### Manufacture

In general, DCHP is manufactured commercially in a closed system by catalytically esterifying phthalic anhydride with cyclohexane ring alcohols (cyclohexanol). As with other phthalates, the unreacted alcohols are recovered and reused, and the DCHP mixture is purified by vacuum distillation or activated charcoal. The purity of DCHP can achieve 99% or greater using current manufacturing processes. The remaining fraction of DCHP may contain a maximum of 0.1% water and 0.15% phthalic acid.

Bayer Polymers, LLC manufactures Unimoll 66/66M, a dicyclohexyl phthalate product used to improve the storage stability and fusing characteristics of vinyl plastisols. Other products are manufactured and sold without trade names as "dicyclohexyl phthalate".

### Supply

U.S. production of DCHP is low and has been combined with several other phthalates (benzyl, undecyl dodecyl, n-butyl cyclohexyl, n-butyl-2-ethylhexyl, diisobutyl, 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 DCHP is low and has been combined with several other phthalates (undecyl dodecyl, n-butyl cyclohexyl, n-butyl-2-ethylhexyl, diisobutyl, isooctyl isodecyl, diethylene glycol, isooctyl diphenyl, cyclohexyl-2-ethylhexyl, and di-(butoxyethyl) phthalate) in marketing reports (Bizzari et al. 2009). Historically, combination 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 DCHP has been slightly higher than production, meaning that DCHP produced in the U.S. is probably utilized locally and also that a small amount of DCHP may be imported.

Production volumes reported in the Hazardous Substance Data Bank (HSDB) for DCHP indicate that the production volume range was >500 thousand - 1 million pounds in 2002 (HSDB, 2009). These production volumes are for non-confidential chemicals reported under the U.S. EPA Inventory Update Rule.

Use

Transitional phthalates esters are used primarily as industrial chemicals that are associated with polymers to impart flexibility in polyvinyl chloride (PVC) resins. DCHP is generally used as a plasticizer for cellulose nitrate, benzyl cellulose, ethyl cellulose, chlorinated rubber, polyvinyl acetate, polyvinyl butyral, PVC, polystyrene, acrylic plastics when products are intended for food or drink contact, and other polymers (NICNAS, 2008). DCHP is also used as a heat sealer for cellulose and in paper finishes (i.e. food wrappers/labels, pharmaceutical labels, price labels; HSDB, 2009). NICNAS (2008) reported that in Australia, DCHP is imported for adhesive manufacture (i.e. hot melt adhesives, sometimes as high as 60% by volume; underfloor sealing compounds) and use in screen printing inks for paper, vinyl, textiles, and other substrates. DCHP is also used as an additive to retard the oxidation of peroxides.

Specifically, the U.S. FDA has approved DCHP for use: in the manufacture of cellophane from food packaging alone, or in combination with other phthalates where total phthalates do not exceed 5% (21 CFR part 177.1200), as a component in coated or uncoated food-contact surface of paper and paperboard used for all aspects of handling aqueous or fatty foods (21 CFR part 176.120), as a component of adhesives for food contact articles (21 CFR part 175.105), in polymeric substances used in all aspects of food handling (21 CFR part 178.3740), and in plastic

film (at concentrations of < 10% total phthalates) prepared from polyvinylacetate, polyvinyl chloride, and vinyl chloride copolymers.

## 4. TOXICOKINETICS

Primate, rat, and ferret hepatic and intestinal preparations and rat gastrointestinal contents have been shown to hydrolyze dicyclohexyl phthalate (DCHP) in vitro to its corresponding monoester, monocyclohexyl phthalate (MCHP) (Lake et al., 1977; Rowland et al., 1977). Based on data for rats, the rate of DCHP metabolism is considerably slower using stomach and cecum contents (3.8 and 5 times slower, respectively) than using the contents of the small intestine. Human feces only inefficiently catalyzed the hydrolysis of DCHP to MCHP. Given that rates of DCHP hydrolysis were low for rat cecum contents and human feces in comparison to rat small intestine contents, enzymes of mammalian, and not bacterial, origin are implicated in DCHP metabolism. Rates of DCHP hydrolysis appear to vary by species; baboon hepatic and intestinal preparations were approximately 5- and 13-fold more efficient, respectively, than similar preparations from rats and ferrets. In comparison to the metabolism of other examined phthalate diesters (dimethyl phthalate [DMP], diethyl phthalate [DEP], and dibutyl phthalate [DBP]) to their corresponding monoesters, metabolism of DCHP to MCHP occurred at a several-fold slower rate (depending on the preparation used). These data suggest that ingestion of DCHP via the oral route results in intestinal absorption of its monoester derivative; the toxicity of DCHP is likely related to its rate of hydrolysis to MCHP and the properties of MCHP.

Bovine and porcine pancreatic cholesterol esterases (CEases; 50 U) were able to completely metabolize (5 µmole) DCHP to MCHP in vitro within 24 hours (Saito et al., 2010). In contrast, a bacterial CEase from *Psuedomonas aeruginosa* was not able to hydrolyze DCHP. Although sequential hydrolyses of phthalate diesters to their corresponding monoesters and then phthalic acid (PA) is thought to occur, PA was not formed by DCHP metabolized by bovine or porcine pancreatic CEases. Compared to the time course of hydrolysis for other phthalate diesters (including DEP, di-n-propyl phthalate [DPrP], DBP, di-n-pentyl phthalate [DPeP], and di-n-hexyl phthalate [DHP; straight chain] or di-2-ethylhexyl phthalate [DEHP; branched chain], hydrolysis of DCHP (which contains a cyclic alkyl chain) was slower (other phthalates completely hydrolyzed in 12 minutes compared to 6 hours for DCHP [DEP, DPrP, DBP, DPeP > DHP ≥ DEHP > BBP >> DCHP]). The authors suggested that the rate of hydrolysis of DCHP is affected by the bulkiness of the alkyl side chains of DCHP.

No data were located on absorption or elimination kinetics of DCHP.

## 5. HAZARD INFORMATION

This section contains brief hazard summaries of the adverse effects of DCHP 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 DCHP resulted in oral $LD_{50}$s >3200 mg/kg in four animal studies. Slight dermal irritation was noted in one well-described guinea pig study and mild dermal irritation was reported in a rabbit study. Slight eye irritation was reported in a rabbit study. DCHP was also reported to not be a sensitizer in guinea pigs by one information source. Insufficient data were available to make the determination of whether DCHP was associated with acute dermal or inhalation toxicity.

Evidence supported the conclusion that DCHP was a subchronic toxicant. Exposure to DCHP induced changes in body weight, liver, kidney, and thyroid weight and pathology, and reproduction and development (mean estrous cycle, homogenization resistant sperm, anogenital distance, areola without nipples, resorptions, live fetuses, number of pups born, reproductive organ weights, incidence of cervical ribs, and incidence of hypospadias) following subchronic to multigenerational administration.

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 DCHP relevant exposure durations 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 DCHP (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

The median lethal oral dose ($LD_{50}$) was reported to be >3,200 mg/kg in rats and mice in studies by Eastman Kodak Co. (1965). Bornmann et al. (1956, as cited in European Commission, 2000 and Lefaux, 1968) reported $LD_{50}$ values of 30 mL/kg in rats and >15 mL/kg in rabbits and dogs for a solution containing 25% DCHP in olive oil (approximately 10,375[1] and >5,188 mg DCHP/kg, respectively, using the reported density of 1,383 kg/$m^3$ for DCHP [NICNAS, 2008]). In guinea pigs, an acute oral $LD_{50}$ value of >20,000 mg/kg was reported (DuPont, 1982, as cited in NICNAS, 2008). No further details were available for any of these studies.

[1]30 mL solution/kg body weight × 0.25 mL DCHP/mL solution = 7.5 mL DCHP/kg body weight; 7.5 mL DCHP/kg body weight × 1,383 mg DCHP/mL DCHP = 10,375 mg DCHP/kg body weight.

The estimated $LD_{50}$'s from the Bornmann et al. (1956) and DuPont (1982) studies in rabbits, dogs, and guinea pigs were higher than the oral $LD_{50}$ range (50–5,000 mg/kg) required by the FHSA to conclude that a chemical is acutely toxic. In addition, Eastman Kodak Co. (1965) reported an $LD_{50}$ of >3,200 mg/kg in rats and mice (presumably 3,200 mg/kg was the highest dose tested).

The weight of evidence including probable animal data are sufficient, therefore, to support the conclusion that DCHP does not fit the definition of "acutely toxic" via oral exposure under the FHSA (16 CFR §1500.3(c)(2)(i)(A)).

### 5.2. Acute Dermal Toxicity

Acute dermal toxicity studies of DCHP were not identified; however, one study was located that evaluated exposure to a mixture containing DCHP. No mortality was reported in albino rabbits (three males and three females) exposed to 2,000 mg/kg of Nuoplaz 6938, a mixture composed of 61.2% n-butyl cyclohexyl phthalate, 15.2% DCHP, 21.9% DBP, and 1.7% DMP by weight, via the dermal route (clipped, abraded skin under occluded conditions) for 24 hours and observed for 14 days. An $LD_{50}$ value of >2,000 mg/kg was identified for the mixture (Nuodex, Inc., 1979a).

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

### 5.3. Acute Inhalation Toxicity

No acute inhalation toxicity studies of DCHP were located. An $LC_{50}$ value of >20.8 mg/L (>20,800 mg/m$^3$) was identified in Wistar rats (five males and five females) whole-body exposed to the Nuoplaz 6938 mixture for 1 hour and observed 14 days after dosing (Nuodex, Inc., 1979b). There were no deaths in this study.

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

### 5.4. Primary Skin Irritation

Mild irritation was seen following repeated 4-hour applications of DCHP to the skin of rabbits (Timofievskaya et al., 1981, as cited in NICNAS, 2008). Eastman Kodak Co. (1965) reported that DCHP was a slight skin irritant in guinea pigs. No further information was located for either of these studies.

Albino rabbits (n = 6) administered 500 mg of the Nuoplaz 6938 mixture to intact or abraded skin for 24 hours (under occluded conditions) showed no erythema or edema (Nuodex, Inc., 1979c). Four of 10 male guinea pigs repeatedly exposed to 500 mg Nuoplaz 6938 on intact skin under the same conditions (for 10 applications) showed erythema and slight edema at the test site following applications 3 through 10 (Nuodex, Inc., 1979d).

The weight of evidence including sufficient animal data supported the conclusion that DCHP did not fit the definition of a dermal "corrosive" as outlined in the FHSA (16 CFR §1500.3(c)(3)).

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

### 5.5. Primary Eye Irritation

DCHP elicited slight irritation (without corneal damage) to the eyes of rabbits (Eastman Kodak Co., 1965). No further details were provided. Nuoplaz 6938 administered to the right eyes of rabbits (n = 6), caused moderate irritation of the conjunctivae (including redness, chemosis, and discharge), but no irritation to the cornea or iris, 1 and 2 days after instillation; no irritation was detected in treated eyes by day 3 post-instillation (Nuodex, Inc., 1979e).

The weight of evidence including sufficient animal data supported the conclusion that DCHP 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 properties of DCHP (by itself) can be considered a data gap and supports the conclusion that there is "inadequate

evidence" for the designation of DCHP as an ocular "primary irritant" when considering FHSA criteria (16 CFR §1500.3(c)(3)).

### 5.6. Sensitization

Eastman Kodak Co. (1965) reported that DCHP was not a skin sensitizer in guinea pigs. No further information was available.

Male guinea pigs were repeatedly exposed to 500 mg Nuoplaz 6938 on intact skin for 24 hours (under occluded conditions) for 10 applications and re-challenged at a different site after a 2-week rest period. Four of 10 animals showed erythema and slight edema 24 and 48 hours after the challenge application (Nuodex, 1979d).

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

### 5.7. Respiratory Sensitization

A meat worker (male, 58 years old) who experienced wheezing when working with heated labels showed signs of respiratory distress (characterized by tightness in the chest, wheezing, and decreased spirometry parameters) upon challenge with heated label emissions for 4 minutes (Levy, 1978). Label emissions contained DCHP, phthalic anhydride, and 2,5-di-tert-amyl-quinone. No significant changes in spirometry measurements were observed upon re-challenge 1 month later.

## REPEAT DOSE TOXICITY

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

Gavage studies using a preparation containing 25% DCHP in olive oil found no effects in rats treated with 2 mL/kg (approximately 700 mg/kg DCHP) twice a week for 6 weeks or 0.5 or 1 mL/kg (approximately 175 or 350 mg/kg DCHP) twice weekly for 52 weeks and observed for an additional 3 months (Bornmann et al., 1956, as cited in NICNAS, 2008, European Commission, 2000, and Lefaux, 1968).

SPF albino rats (10/sex/group) administered DCHP (purity not known) at 0, 0.05, 0.15, 0.4, or 1% in the diet for 90 days (corresponding to doses of 0, 25, 75, 200, and 500 mg/kg-day as cited in European Commission, 2000) showed no mortality or clinical signs of toxicity (de Ryke and Willems, 1977, as cited in NICNAS, 2008 and European Commission, 2000). Effects reported by European Commission (2000) included decreased body weight gain and food consumption in males at 500 mg/kg-day. In a second, follow-up study (de Ryke and Bosland, 1978, as cited in European Commission, 2000), albino rats (10/sex/group) were administered DCHP at 0, 0.075, 0.1, 0.15, or 1% in the diet for 90 days (0, 37.5, 50, 75, or 500 mg/kg-day). As in the first study, no mortality or clinical signs of toxicity were observed. European Commission (2000) reported decreased body weight and food intake in males, but it is not clear from the study whether these effects were seen in both the 75 and 500 mg/kg-day groups or only the 500 mg/kg-day group. Available information on these studies was insufficient for independent assessment.

No effects were reported in rats treated with DCHP at 27 mg/kg-day or in dogs treated with DCHP at 14 mg/kg-day in 2- and 1-year feeding studies, respectively (Shibko and Blumenthal, 1973). No further information was available.

In a preliminary dose-range finding study, Sprague-Dawley rats (group sizes not reported) were administered DCHP in the diet at 0, 600, 2,000, 6,000, or 20,000 ppm during the period from 3 weeks or more before mating, through mating, and until necropsy (males) or through gestation and lactation periods (females) (Hoshino et al., 2005). Parameters evaluated were not explicitly specified. However, the researchers reported inhibition of body weight gain in rats administered the high dose of 20,000 ppm. In the main two-generation reproductive toxicity study that followed, groups of 24 male and 24 female Sprague-Dawley rats were administered DCHP in the diet at 0, 240, 1,200, or 6,000 ppm (Hoshino et al., 2005). Doses of 0, 16–21, 80–107, and 402–534 mg/kg-day were calculated by the study authors. Dosing of F0

parents was initiated at 5 weeks of age and continued through pre-mating and mating (males) or pre-mating, mating, gestation, and lactation periods (females). F1 animals were administered DCHP from the time of weaning (postnatal day [PND] 21) and through the same periods of pre-mating, mating, gestation, and lactation. Parental animals (F0 females and F1 males) showed significant inhibition of body weight gain; final body weights of F0 females were 5–8% lower and F1 males were 10–12% lower at 1,200 and 6,000 ppm than concurrent controls (Table 5.1). Food consumption was likewise reduced in the same parental groups. No-observed-adverse effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) values based on decreased body weight in this study were 240 and 1,200 ppm (corresponding to 18–21 and 90–104 mg/kg-day, respectively, in the affected groups [F0 females and F1 males]).

**Table 5.2. Body Weight Data for Sprague-Dawley Rats Administered DCHP in the Diet Prior to Mating, Through Mating, and Until Necropsy (Males) or Through Gestation and Lactation Periods (Females)**

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 240 | 1,200 | 6,000 |
| **Parental animals** | | | | |
| Body weight at study termination (g) | | | | |
| F0 males | 510.5 ± 50.4[a] | 503.6 ± 42.5 | 500.4 ± 28.0 | 496.7 ± 37.1 |
| F0 females | 322.1 ± 19.7 | 311.9 ± 16.6 | 306.5 ± 18.2[b] | 295.3 ± 18.8[c] |
| F1 males | 624.9 ± 48.9 | 603.4 ± 54.0 | 564.7 ± 42.0[c] | 552.5 ± 30.5[c] |
| F1 females | 337.4 ± 27.8 | 338.7 ± 23.9 | 330.8 ± 18.8 | 320.7 ± 22.7 |

[a]Mean ± standard deviation (SD).
[b]Significantly different from controls at $p < 0.05$ (as reported by the study authors).
[c]Significantly different from controls at $p < 0.01$.

Source: Hoshino et al. (2005).

No mortality or clinical signs of toxicity were observed in time-mated pregnant Sprague-Dawley rats (24–25 group) administered DCHP via gavage at 0, 250, 500, or 750 mg/kg-day on gestation days (GDs) 6–20 and sacrificed on GD 21 (Saillenfait et al., 2009). However, rats treated with DCHP at the high dose of 750 mg/kg-day showed significant decreases in body weight gain (22%), body weight (12%), and food consumption relative to controls (Table 5.2). Rats in the mid-dose group also experienced transitory decreases in food consumption and body weight gain early in gestation.

| Table 5.3. Body Weight and Food Consumption Data for Sprague-Dawley Rats Administered DCHP via Gavage on GDs 6–20 | | | | |
|---|---|---|---|---|
| | Dose (mg/kg-day) | | | |
| Parameter | 0 | 250 | 500 | 750 |
| **Parental animals** | | | | |
| Body weight change (g) | | | | |
| GDs 6–9 | 15 ± 5[a] | 13 ± 5 | 10 ± 5[b] | 8 ± 8[c] |
| GDs 18–21 | 48 ± 14 | 50 ± 9 | 38 ± 17 | 26 ± 18[c] |
| GDs 6–21 | 145 ± 27 | 146 ± 19 | 129 ± 32 | 113 ± 30[c] |
| Body weight (g) | | | | |
| GD 21[d] | 337 ± 22 | 327 ± 20 | 314 ± 21 | 297 ± 20[c] |
| Food consumption (g/day) | | | | |
| GDs 6–9 | 20 ± 3 | 19 ± 3 | 17 ± 2[c] | 16 ± 3[c] |
| GDs 18–21 | 22 ± 4 | 23 ± 3 | 21 ± 5 | 18 ± 5[c] |

[a]Mean ± SD.
[b]Significantly different from controls at $p < 0.05$.
[c]Significantly different from controls at $p < 0.01$.
[d]Excluding uterine weight.

Source: Saillenfait et al. (2009).

Yamasaki et al. (2009) administered DCHP at 0, 20, 100, or 500 mg/kg-day to timed-pregnant Crl:CD(SD) IGS rats (10/group) via gavage on GDs 6–20 and allowed the dams to give birth. One female treated with DCHP at 500 mg/kg-day exhibited dystocia and died on GD 23. Body weights of treated rats were not significantly different from controls.

## 5.9. Hepatotoxicity

Lake et al. (1982) found evidence for liver effects in male Sprague-Dawley rats administered DCHP via gavage at 0, 500, 1,000, 1,500, or 2,500 mg/kg-day for 7 days. A dose-related increase in relative liver weight was observed in DCHP-treated rats; relative liver weight was increased by 42% in rats treated with 1,500 mg/kg-day DCHP compared to vehicle-only controls (Table 5.3; numerical data for other dose groups not provided in study report). Significant induction of several enzymes, including 7-ethoxycoumarin 0-deethylase (288% of control), biphenyl 4-hydroxylase (234% of control), and aniline 4-hydroxylase (128% of control), and increased levels of microsomal cytochrome P450 (139% of control), cytochrome $b_5$ (118% of control), and heme (125% of control) were also seen in the livers of rats treated with DCHP at 1,500 mg/kg-day. Based on data presented graphically, relative liver weight, 7-ethoxycoumarin 0-deethylase activity, and hepatic cytochrome P450 content appeared to differ substantially from controls at doses <1,500 mg/kg-day (and as low as 500 mg/kg-day; statistical

analyses not performed). Slight hypertrophy of centrilobular cells of the liver was noted at 1,500 mg/kg-day; this effect was more marked in rats dosed with 2,500 mg/kg-day DCHP. Ultrastructural analyses showed that treatment with 1,500 mg/kg-day DCHP led to marked proliferation of the smooth endoplasmic reticulum of centrilobular cells of the liver lobule; other intracellular organelles looked similar to controls. There was no evidence of peroxisome proliferation. In summary, treatment with DCHP elicited hepatic enzyme induction, increased relative liver weight, proliferation of smooth endoplasmic reticulum, and hepatocellular hypertrophy at 1,500 mg/kg-day in this study, with some of these effects apparently occurring at doses as low as 500 mg/kg-day.

**Table 5.4. Liver Effects in Male Sprague-Dawley Rats Administered DCHP via Gavage for 7 Days**

| | Dose (mg/kg-day) | |
|---|---|---|
| | **0** | **1,500** |
| Relative liver weight (g/100 g body weight) | 3.3 ± 0.1[a] | 4.7 ± 0.1 (142)[b,c] |
| Hepatic enzyme levels | | |
| 7-Ethoxycoumarin-0-deethylase (µmol/hour/g liver) | 5.8 ± 0.4 | 16.7 ± 1.3 (288)[c] |
| Biphenyl 4-hydroxylase (µmol/hour/g liver) | 8.7 ± 0.6 | 20.4 ± 1.3 (234)[c] |
| Aniline 4-hydroxylase (µmol/hour/g liver) | 4.3 ± 0.3 | 5.5 ± 0.3 (128)[d] |
| Cytochrome P450 (nmol/mg microsomal protein) | 1.04 ± 0.04 | 1.45 ± 0.02 (139)[c] |
| Cytochrome $b_5$ (nmol/mg microsomal protein) | 0.40 ± 0.02 | 0.47 ± 0.02 (118)[d] |
| Microsomal haem (nmol/mg microsomal protein) | 1.53 ± 0.05 | 1.91 ± 0.04 (125)[c] |

[a]Mean ± standard error of the mean (SEM) for groups of 6–12 animals.
[b]Percentage of control values shown in parentheses.
[c]Significantly different from controls at $p < 0.001$.
[d]Significantly different from controls at $p < 0.05$.

Source: Lake et al. (1982).

Consistent with the results of Lake et al. (1982), liver enlargement was among the effects reported in a 21-day gavage study in which rats were treated with 4,170 mg/kg-day of DCHP (Grasso, 1978, as cited in NICNAS, 2008). No further details were provided.

SPF albino rats (10/sex/group) administered DCHP at 0, 0.05, 0.15, 0.4, or 1% (0, 25, 75, 200, or 500 mg/kg-day) in the diet for 90 days also showed evidence of hepatic effects (de Ryke and Willems, 1977, as cited in NICNAS, 2008 and European Commission, 2000). Effects reported by European Commission (2000) included increased serum alkaline phosphatase in males at ≥25 mg/kg-day and females at 500 mg/kg-day, increased relative liver weight in males at ≥200 mg/kg-day and females at ≥75 mg/kg-day, and unspecified histopathological changes to the liver at ≥200 mg/kg-day. In contrast, NICNAS (2008) reported that the study found liver

weight changes, but no histopathology, at 200 mg/kg-day. The parameters that were affected by DCHP treatment were re-evaluated in a second study (de Ryke and Bosland, 1978, as cited in European Commission, 2000). In the follow-up study, albino rats (10/sex/group) were administered DCHP at 0, 0.075, 0.1, 0.15, or 1% in the diet for 90 days (0, 37.5, 50, 75, or 500 mg/kg-day). Effects reported by European Commission (2000) included increased serum alkaline phosphatase, increased relative liver weight, and minimal histopathological changes (unspecified) in the liver in both sexes. It is not clear from the reporting in European Commission (2000) whether these effects were seen in both the 75 and 500 mg/kg-day groups or only the 500 mg/kg-day group. Available information on these studies was insufficient for independent assessment.

In the preliminary dose-range finding study for the rat two-generation dietary reproduction study (Hoshino et al., 2005), the researchers reported increased liver weights in rats administered DCHP at ≥2,000 ppm (data not shown; absolute or relative weights not specified). In the main two-generation reproductive toxicity study (Hoshino et al., 2005), absolute and/or relative liver weights were significantly increased at 6,000 ppm in all parental groups (Table 5.4). Relative liver weight was also significantly increased in F0 females at 1,200 ppm. Hypertrophy of hepatocytes (diffuse) was observed in 1,200- and 6,000-ppm animals; the incidence of hypertrophy was significantly increased compared to controls at 6,000 ppm. From these data, NOAEL and LOAEL values of 240 and 1,200 ppm are identified for liver effects based on increased relative liver weight in F0 females (doses of 21 and 104 mg/kg-day in this group).

| Table 5.5. Liver Effects in Sprague-Dawley Rats Administered DCHP in the Diet | | | | |
|---|---|---|---|---|
| | Dose (ppm) | | | |
| **Parameter** | **0** | **240** | **1,200** | **6,000** |
| **Parental animals** | | | | |
| F0 adults | | | | |
| Absolute liver weight (g) | 15.042 ± 1.911[a,b]<br>10.233 ± 0.944[c] | 14.620 ± 1.960<br>9.907 ± 0.772 | 14.596 ± 1.183<br>10.298 ± 0.824 | 18.157 ± 1.730[d]<br>11.157 ± 0.995[d] |
| Relative liver weight (percent body weight) | 2.944 ± 0.203[b]<br>3.174 ± 0.168[c] | 2.898 ± 0.229<br>3.176 ± 0.171 | 2.917 ± 0.177<br>3.362 ± 0.190[e] | 3.658 ± 0.252[d]<br>3.779 ± 0.251[d] |
| Liver: hypertrophy, hepatocytes, diffuse | 0/24[b]<br>0/24[c] | 0/24<br>0/24 | 4/24<br>3/24 | 16/24[f]<br>12/24[f] |
| F1 adults | | | | |
| Relative liver weight (percent body weight) | 3.00 ± 0.210[b]<br>3.78 ± 0.455[c] | 2.88 ± 0.195<br>3.64 ± 0.360 | 2.94 ± 0.238<br>3.90 ± 0.467 | 3.42 ± 0.314[d]<br>4.39 ± 0.393[d] |
| Liver: hypertrophy, hepatocytes, diffuse | 0/20[b]<br>0/20[c] | 0/23<br>0/23 | 0/20<br>0/20 | 14/22[f]<br>9/22[f] |

[a]Mean ± SD.
[b]Males.
[c]Females.
[d]Significantly different from controls at $p < 0.01$ (as reported by the study authors).
[e]Significantly different from controls at $p < 0.05$.
[f]$p < 0.05$ compared to control group using Fisher's exact test (performed for this evaluation).

Source: Hoshino et al. (2005).

In the Saillenfait et al. (2009) gestational exposure study, relative, but not absolute, liver weights of dams were significantly higher than those of untreated controls at 500 and 750 mg/kg-day (17 and 28% higher, respectively; Table 5.5). The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were elevated only in high-dose rats; mild but significant (1.7–2.1-fold) induction of hepatic peroxisomal B oxidation activity was observed in all treatment groups. Levels of cholesterol and triglycerides in the serum of treated rats were comparable to controls. No histopathological changes attributable to DCHP treatment were observed. Based on these data, NOAEL and LOAEL values of 250 and 500 mg/kg-day (for increased relative liver weight) are identified for liver effects.

| Table 5.6. Liver Effects in Sprague-Dawley Rats Administered DCHP via Gavage on GDs 6–20 | | | | |
|---|---|---|---|---|
| | Dose (mg/kg-day) | | | |
| **Parameter** | **0** | **250** | **500** | **750** |
| **Parental animals** | | | | |
| AST (U/L) | 61 ± 6[a] | 76 ± 16 | 76 ± 14 | 91 ± 19[b] |
| ALT (U/L) | 46 ± 8 | 53 ± 6 | 57 ± 13 | 101 ± 18[b] |
| Relative liver weight (percent body weight) | 4.22 ± 0.44 | 4.62 ± 0.38 | 4.95 ± 0.33[b] | 5.40 ± 0.46[b] |
| Hepatic palmitoyl coA oxidase activity (nmol/min/mg proteins) | 14.2 ± 2.8 | 24.8 ± 5.3[b] | 27.0 ± 5.3[b] | 29.6 ± 1.7[b] |

[a]Mean ± SD.
[b]Significantly different from controls at $p < 0.01$.

Source: Saillenfait et al. (2009).

The gestational exposure study by Yamasaki et al. (2009) reported increased absolute and relative liver weights at 100 and 500 mg/kg-day. Data for absolute liver weight were not shown. Relative liver weight was increased by 7 and 24% at 100 and 500 mg/kg-day, respectively (Table 5.6). Based on these data, NOAEL and LOAEL values of 20 and 100 mg/kg-day (for increased relative liver weight) are identified for liver effects in this study.

| Table 5.7. Liver Effects in Crl:CD(SD) IGS Rats Treated with DCHP via Gavage on GDs 6–20 | | | | |
|---|---|---|---|---|
| | Dose (mg/kg-day) | | | |
| **Parameter** | **0** | **20** | **100** | **500** |
| Relative liver weight (percent body weight) | 4.65 ± 0.29[a] | 4.82 ± 0.32 | 4.97 ± 0.25[b] | 5.75 ± 0.17[b] |

[a]Mean ± SD.
[b]Significantly different from controls at $p < 0.05$.

Source: Yamasaki et al. (2009).

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

## 5.10. Renal Toxicity

No changes in relative kidney weight or kidney histopathology were observed in male Sprague-Dawley rats administered DCHP via gavage at up to 2,500 mg/kg-day for 7 days (Lake et al., 1982).

Effects reported by European Commission (2000) in rats administered DCHP at up to 1% (500 mg/kg-day) in the diet for 90 days (de Ryke and Willems, 1977) included unspecified histopathological changes to the kidney at ≥200 mg/kg-day. However, NICNAS (2008) reported no histopathology at 200 mg/kg-day in this study. In a second, follow-up study (de Ryke and Bosland, 1978, as cited in European Commission, 2000), no kidney effects were reported in rats administered DCHP at up to 1% in the diet (500 mg/kg-day) for 90 days.

In the two-generation dietary reproductive toxicity study (Hoshino et al., 2005), parental male rats (both F0 and F1) showed increased severity of hyaline droplets in the renal proximal tubular epithelium at the high-dose of 6,000 ppm (Table 5.7). Based on these data, NOAEL and LOAEL values of 1,200 and 6,000 ppm (80–90 and 402–457 mg/kg-day in F0/F1 males) are identified for histopathological changes in the kidney (increased severity of hyaline droplet formation).

**Table 5.8. Kidney Effects in Sprague-Dawley Rats Administered DCHP in the Diet**

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | **0** | **240** | **1,200** | **6,000** |
| F0 adults | | | | |
| Kidney: hyaline droplet, proximal tubular epithelium | | | | |
| Slight | 23/24[a]<br>0/24[b] | 22/24<br>Not examined | 23/24<br>0/1 | 9/24<br>0/24 |
| Moderate | 1/24[a]<br>0/24[b] | 1/24<br>Not examined | 0/24<br>0/1 | 15/24[c]<br>0/24 |
| F1 adults | | | | |
| Kidney: hyaline droplet, proximal tubular epithelium | | | | |
| Slight | 19/20[a]<br>0/20[b] | 22/23<br>Not examined | 20/20<br>Not examined | 14/22<br>0/22 |
| Moderate | 1/20[a]<br>0/20[b] | 1/23<br>Not examined | 0/20<br>Not examined | 8/22[c]<br>0/22 |

[a]Males.
[b]Females.
[c]$p < 0.05$ compared to control group using Fisher's exact test (performed for this evaluation).

Source: Hoshino et al. (2005).

## 5.11. Gastrointestinal Toxicity

In a 21-day gavage study, rats treated with DCHP at 4,170 mg/kg-day reportedly exhibited squamous cell hyperplasia in the stomach (Grasso, 1978, as cited in NICNAS, 2008). No other details were provided.

## 5.12. Endocrine Activity

In the two-generation reproductive toxicity study (Hoshino et al., 2005), hypertrophy of thyroid follicular epithelial cells was observed in 1,200- and 6,000-ppm animals; the incidence of hypertrophy was significantly increased at 6,000 ppm compared to controls (Table 5.8). Absolute and relative thyroid weights were significantly increased in F0 adults at 6,000 ppm. NOAEL and LOAEL values of 1,200 and 6,000 ppm (80–107 and 402–534 mg/kg-day) are identified for increased thyroid weight and hypertrophy of thyroid follicular epithelial cells.

**Table 5.9. Significant Thyroid Effects in Sprague-Dawley Rats Administered DCHP in the Diet**

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 240 | 1,200 | 6,000 |
| F0 adults | | | | |
| Absolute thyroid weight (left) (mg) | 11.33 ± 2.65[a,b]<br>10.08 ± 2.15[c] | 12.48 ± 2.47<br>9.74 ± 1.59 | 11.99 ± 2.11<br>9.91 ± 1.80 | 14.37 ± 4.02[d]<br>11.35 ± 1.39 |
| Relative thyroid weight (left) (percent body weight × 1,000) | 2.23 ± 0.51[b]<br>3.13 ± 0.68[c] | 2.48 ± 0.46<br>3.12 ± 0.49 | 2.40 ± 0.43<br>3.23 ± 0.57 | 2.91 ± 0.91[d]<br>3.87 ± 0.54[d] |
| Thyroid: hypertrophy, follicular cells | 0/24[b]<br>0/24[c] | 0/24<br>0/24 | 3/24<br>0/24 | 7/24[e]<br>6/24[e] |
| F1 adults | | | | |
| Thyroid: hypertrophy, follicular cells | 0/20[b]<br>0/20[c] | 0/23<br>0/23 | 0/20<br>0/20 | 7/22[e]<br>6/22[e] |

[a]Mean ± SD.
[b]Males.
[c]Females
[d]Significantly different from controls at $p < 0.01$.
[e]$p < 0.05$ compared to control group using Fisher's exact test (performed for this evaluation).

Source: Hoshino et al. (2005).

Endpoints associated with the estrous cycle and levels of sex hormones in the serum are discussed in Section 5.13, Reproductive Toxicity. Endpoints associated with sexual maturation of pups are discussed in Section 5.14, Developmental Toxicity.

The weight of evidence from the above studies supported the conclusion that there was "limited animal evidence" for the designation of DCHP as a "thyroid toxicant".

### 5.13. Reproductive Toxicity

Estrous cycle and fertility were reportedly unaffected in female rats (8/group) administered 25% DCHP in olive oil at 2 mL/kg (approximately 700 mg/kg-day) by daily gavage for 6 weeks and then mated to untreated males (Bornmann et al., 1956, as cited in NICNAS, 2008 and European Commission, 2000). Two subsequent generations of rats (untreated and inbred) also showed no impairments in fertility (F1 and F2 adults). Reproduction was "normal" with "no anomalies...in parturition or nursing" in Wistar rats administered DCHP at 100 ppm in the diet for 18 months over four generations (Lefaux, 1968). The dose was estimated as 5 mg/kg-day by NICNAS (2008). No additional study details were provided.

No changes in relative testes weights were observed in male Sprague-Dawley rats administered DCHP via gavage at 0, 500, 1,000, 1,500, 2,000, or 2,500 mg/kg-day for 7 days (Lake et al., 1982). In this study, one of five animals treated with 2,500 mg/kg-day DCHP exhibited bilateral tubular atrophy of 30–40% of the germinal cells of the testes. None of the other examined animals showed any abnormalities in the testes, however. Testicular atrophy was cited as an effect in a study in which rats were administered DCHP at 4,170 mg/kg-day via gavage for 21 days (Grasso, 1978, as cited in NICNAS, 2008). No other details were provided.

Testicular atrophy was also reported in the dietary two-generation study in rats (Hoshino et al., 2005). Histological examination showed atrophy of the seminiferous tubules in mid- and high-dose F1 males. In the high-dose group, the incidence of the lesion was significantly increased relative to controls and the lesion was graded as severe in several animals (Table 5.9). Also in the F1 parental males, spermatid head counts in the testes were significantly decreased in the 1,200- and 6,000-ppm group by 15 and 24%, respectively, compared to controls (Table 5.9). Other sperm parameters were unaffected in F1 males. No sperm or testicular changes were seen in F0 parental males. Absolute prostate weights were reduced in F1 males at all doses relative to controls, but relative prostate weight was decreased only at the high dose. Prostate weights were unchanged from controls in the F0 males. The only effect in females was a slight increase in estrous cycle length at the high dose in the F0 generation. No effects were observed on serum hormone levels in males or females or reproductive capability (mating, fertility, gestation, and birth index). NOAEL and LOAEL values of 240 and 1,200 ppm (18 and 90 mg/kg-day) are identified for reproductive toxicity in F1 adult males based on seminiferous tubule atrophy and

significant reductions in spermatid head counts in this group. The high dose of 6,000 ppm (511–534 mg/kg-day) is a NOAEL for reproductive effects in female rats in this study.

**Table 5.10. Reproductive Effects in Sprague-Dawley Rats Administered DCHP in the Diet**

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 240 | 1,200 | 6,000 |
| F0 females | | | | |
| Estrous cycle length (days) | 4.04 ± 0.14[a] | 4.06 ± 0.22 | 4.00 ± 0.00 | 4.25 ± 0.42[b] |
| F1 males | | | | |
| Spermatid head count ($10^6$/g) | 104.0 ± 12.66 | 93.4 ± 10.27 | 88.6 ± 10.32[b] | 79.2 ± 30.29[c] |
| Absolute prostate weight (g) | 0.71 ± 0.152 | 0.58 ± 0.133[c] | 0.59 ± 0.149[b] | 0.51 ± 0.118[c] |
| Relative prostate weight (percent body weight) | 0.11 ± 0.027 | 0.10 ± 0.20 | 0.11 ± 0.029 | 0.09 ± 0.024[b] |
| Testis: atrophy, seminiferous tubules | 1/20 | 0/23 | 2/20 | 9/22[d,e] |

[a]Mean ± SD.
[b]Significantly different from controls at $p < 0.05$ (as reported by the study authors).
[c]Significantly different from controls at $p < 0.01$.
[d]$p < 0.05$ compared to control group using Fisher's exact test (performed for this evaluation).
[e]Graded as severe in three cases.

Source: Hoshino et al. (2005).

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

## 5.14. Prenatal, Perinatal, and Post-natal Toxicity

Development of offspring was reportedly unaffected after female rats (8/group) were administered 25% DCHP in olive oil at 2 mL/kg (approximately 700 mg/kg-day) by daily gavage for 6 weeks and then mated to untreated males (Bornmann et al., 1956, as cited in NICNAS, 2008 and European Commission, 2000). Two subsequent generations of rats (untreated and inbred) also showed no impairments in growth and development (F2 and F3 pups).

In a preliminary dose range-finding study, Hoshino et al. (2005) reported inhibition of body weight gain in the offspring of female Sprague-Dawley rats (group sizes not reported) administered DCHP in the diet at 6,000 and 20,000 ppm prior to mating and throughout mating, gestation and lactation periods. In the main (two-generation) study (Hoshino et al., 2005), significant effects observed in the offspring included inhibition of body weight gain (high-dose

F1 and F2 litters; 4–12%), decreased anogenital distance (AGD) in males (by 7–9%; high-dose F1 pups and mid- and high-dose F2 pups), and increased incidence of areola mammae in males (high-dose F1 and mid- and high-dose F2 pups) (Table 5.10). No adverse effects with regard to other developmental milestones, clinical signs, number of pups delivered, sex ratio of pups, pup viability, reflex and response tests, external abnormalities, or histopathology were observed. NOAEL and LOAEL values of 240 and 1,200 ppm (16–21 and 80–107 mg/kg-day) are identified for developmental toxicity based on decreased AGD and increased incidence of areola mammae in male F1 and F2 offspring.

**Table 5.11. Significant Effects in Sprague-Dawley Rats Administered DCHP in the Diet**

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 240 | 1,200 | 6,000 |
| **Litter data** | | | | |
| Body weights; F1 offspring (g) | | | | |
| Day 0 | 6.8 ± 0.6[a,b]<br>6.5 ± 0.5[c] | 6.8 ± 0.5<br>6.4 ± 0.5 | 6.8 ± 0.4<br>6.4 ± 0.5 | 6.5 ± 0.4[d]<br>6.1 ± 0.4[d] |
| Day 21 | 62.2 ± 4.5[b]<br>59.2 ± 3.7[c] | 61.9 ± 4.8<br>59.6 ± 4.9 | 62.6 ± 4.6<br>59.4 ± 4.7 | 55.0 ± 3.8[e]<br>52.8 ± 3.2[e] |
| Final | 62.49 ± 4.64[b]<br>58.86 ± 3.93[c] | 62.44 ± 5.43<br>59.55 ± 5.39 | 61.71 ± 4.65<br>59.78 ± 5.17 | 55.20 ± 3.65[e]<br>52.27 ± 3.07[e] |
| Body weights; F2 offspring (g) | | | | |
| Day 0 | 6.8 ± 0.4[b]<br>6.4 ± 0.4[c] | 6.6 ± 0.5<br>6.2 ± 0.4 | 6.5 ± 0.5<br>6.2 ± 0.5 | 6.6 ± 0.6<br>6.2 ± 0.6 |
| Day 21 | 64.9 ± 4.2[b]<br>61.7 ± 3.7[c] | 62.8 ± 4.2<br>59.3 ± 3.5 | 62.8 ± 5.0<br>59.2 ± 4.4 | 59.2 ± 5.0[e]<br>56.6 ± 4.3[e] |
| Final | 66.36 ± 3.86[b]<br>60.59 ± 4.94[c] | 62.75 ± 5.30<br>59.70 ± 4.16 | 62.79 ± 6.23<br>59.11 ± 4.67 | 59.93 ± 6.48[e]<br>56.38 ± 4.66[e] |
| Physical development; male F1 offspring | | | | |
| AGD (mm) | 4.68 ± 0.522 | 4.86 ± 0.491 | 4.76 ± 0.448 | 4.37 ± 0.354[e] |
| AGD/body weight$^{1/3}$ | 2.17 ± 0.216 | 2.16 ± 0.213 | 2.11 ± 0.148 | 2.00 ± 0.151[d] |
| Incidence of areole mammae (percent) | 0.0 | 0.0 | 0.0 | 16.1[e] |
| Body weight at preputial separation (g) | 225.3 ± 17.3 | 225.1 ± 12.5 | 218.9 ± 15.4 | 212.5 ± 13.8[d] |
| Physical development; male F2 offspring | | | | |
| AGD (mm) | 4.62 ± 0.314 | 4.49 ± 0.300 | 4.28 ± 0.365[e] | 4.19 ± 0.387[e] |
| AGD/body weight$^{1/3}$ | 2.07 ± 0.152 | 2.02 ± 0.125 | 1.93 ± 0.158[e] | 1.88 ± 0.129[e] |
| Incidence of areola mammae (percent) | 0.0 | 0.0 | 18.4 | 63.2[e] |

[a]Mean ± SD.
[b]Males.
[c]Females.
[d]Significantly different from controls at $p < 0.05$.
[e]Significantly different from controls at $p < 0.01$.

Source: Hoshino et al. (2005).

In the gestational exposure study by Saillenfait et al. (2009), the body weights of male and female pups were decreased in a dose-related manner; an average weight reduction of 11% compared to controls was observed at 750 mg/kg-day (Table 5.11). A significant and dose-related decrease in AGD was seen in all treated males; with respect to controls, AGD was decreased by 9, 12, and 17% in males treated at 250, 500, and 750 mg/kg-day, respectively. The incidences of external, soft tissue, and skeletal malformations and variations in treated rats were comparable to controls. Neither undescended testes nor trans-abdominal testicular migration (TTM) changes were observed in treated rats. A LOAEL of 250 mg/kg-day (and no NOAEL value) is identified for developmental toxicity based on decreased AGD in male fetuses.

**Table 5.12. Significant Changes in the Offspring of Sprague-Dawley Rats Administered DCHP via Gavage on GDs 6–20**

| Parameter | Dose (mg/kg-day) | | | |
|---|---|---|---|---|
| | 0 | 250 | 500 | 750 |
| Fetal body weight (g) | | | | |
| All | 5.63 ± 0.46[a] | 5.51 ± 0.26 | 5.48 ± 0.29 | 5.00 ± 0.47[b] |
| Males | 5.76 ± 0.44 | 5.65 ± 0.25 | 5.61 ± 0.34 | 5.16 ± 0.50[b] |
| Females | 5.45 ± 0.50 | 5.37 ± 0.27 | 5.32 ± 0.30 | 4.85 ± 0.45[b] |
| AGD; males (mm) | 2.98 ± 0.16 | 2.70 ± 0.16[b] | 2.61 ± 0.15[b] | 2.47 ± 0.17[b] |
| AGD/body weight$^{1/3}$; males | 1.66 ± 0.07 | 1.52 ± 0.09[b] | 1.47 ± 0.09[b] | 1.43 ± 0.08[b] |

[a]Mean ± SD.
[b]Significantly different from controls at $p < 0.01$.

Source: Saillenfait et al. (2009).

In the Yamasaki et al. (2009) gestational exposure study, the viability index on PND 4 (percent, number of live pups on PND 4/numer of live pups on PND )[× 100]) was reduced slightly (by 2%) in the 500 mg/kg-day group compared to the control group (Table 5.12). The body weights of pups (males and females) were reportedly decreased significantly at 500 mg/kg-day on PNDs 14 and/or 21 (data not shown). Also with regard to pups, two males from the high-dose group showed hypospadia accompanied by small testes; one of these animals was sacrificed at 7 weeks of age because of poor general condition. With respect to controls, preputial separation was prolonged by 5%, AGD on PND 4 was decreased by 15%, and nipple retention/ incidence of areola was increased by 68% in males treated at 500 mg/kg-day (data for other dose groups not shown); no significant effects with respect to these parameters were reported for the lower dose groups. No abnormalities in vaginal opening day or estrous cycle were observed in treated females. Other than decreased relative muscle weight and slight histological changes (including decreased testicular germ cells and degenerated proximal tubules; incidence data not

shown) in male rats treated at 500 mg/kg-day, treated pups sacrificed at 10 weeks of age were comparable to controls. Significant changes in thymus, spleen, and brain weights in F1 and F2 offspring were attributed by the authors to inhibition of body weight gain, since changes occurred only for either absolute or relative weights, and some showed contrary results between the two.

A developmental LOAEL of 500 mg/kg-day is identified for this study based on slightly reduced viability index (PND 4); decreased weight of male and female pups on PNDs 14/21; and prolonged preputial separation, decreased AGD, increased incidence of areola/nipple retention, and slight histological changes (testis and kidney) in male pups; 100 mg/kg-day is identified as a NOAEL since no adverse effects on these endpoints were reported for this dose group.

**Table 5.13. Significant Effects in Crl:CD(SD) IGS Rats Treated with DCHP via Gavage on GDs 6–20**

| Parameter | Dose (mg/kg-d) | | | |
|---|---|---|---|---|
| | 0 | 20 | 100 | 500 |
| Viability index on PND 4 (percent) | 100.0 ± 0.0[a] | 100.0 ± 0.0 | 100.0 ± 0.0 | 97.8 ± 3.3[b] |
| Age preputial separation (days) | 43.5 ± 2.2 | Not reported | Not reported | 45.6 ± 2.3[b] |
| AGD (mm) | 4.23 ± 0.39 | Not reported | Not reported | 3.59 ± 0.32[b] |
| Incidence areolas/nipples retention (percent) | 0.0 ± 0.0 | Not reported | Not reported | 67.6 ± 40.5[b] |
| Offspring: relative muscle weight (percent body weight) | 0.22 ± 0.02 | 0.20 ± 0.04 | 0.21 ± 0.03 | 0.20 ± 0.02[b] |

[a]Mean ± SD.
[b]Significantly different from controls at $p < 0.05$.

Source: Yamasaki et al. (2009).

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

## 5.15. Carcinogenicity

Genotoxicity

In a test in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 using DCHP (at 100, 333, 1,000, 3,333, or 10,000 µg/plate), DCHP was not shown to be mutagenic in a preincubation modification of the *Salmonella*/microsome assay in the presence or absence of metabolic activation (Zeiger et al., 1985, 1982; NTP, 1983). DCHP did not induce mutation in *Escherichia coli* (wild-type and uvrA- strains) at 30 mg/plate (Kurata, 1975, as cited in Omura et al., 1976). DCHP also tested negative in DNA repair tests in *Bacillus subtilis* (recA-strain) and *E. coli* (uvrA-, polA-, and recA-strains) at 30 mg/plate (Kurata, 1975, as cited in Omori et al., 1976).

Initiation and Promotion

No initiation or promotion studies were located for DCHP.

Carcinogenicity Studies

No carcinogenic effects were observed in Wistar rats administered DCHP at a concentration of 100 ppm (estimated dose of 5 mg/kg-day, as reported in NICNAS, 2008) in the diet in an 18-month, four-generation dietary study (Lefaux, 1968).

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

## 6. EXPOSURE

HSDB (2009) has reported that occupational exposure to dicyclohexyl phthalate may occur through inhalation of aerosols and dermal contact at workplaces where dicyclohexyl phthalate is produced or used. Monitoring and use data indicate that the general population may be exposed via inhalation of ambient air and dermal contact with products containing dicyclohexyl phthalate (HSDB, 2009). The Centers for Disease Control and Prevention (CDC) collects urinary metabolite data for the general U.S. population, primarily through the National Health and Nutrition Examination Survey (NHANES) where metabolites of phthalates have been measured. Reported urinary concentrations for DCHP metabolites have ranged from 0.400 µg/L creatinine ($90^{th}$ percentile) in NHANES 2001-2002 to less than the level of detection ($50^{th}$ percentile) for the total population (CDC, 2005). The specific DCHP associated exposure pathway was not reported.

Migration of DCHP from PVC into potato snacks (DCHP 0.33% of film by weight, 5 days exposure) was 6.2 mg/kg food. Nitrocellulose-coated regenerated cellulose film leached 0.5 to 53 mg DCHP/kg into confectionary, meat pies, cakes, and sandwiches. DCHP also leached from printing inks into food items contacting ink (6% of the total amount of plasticizer transferred). The amount transferred increased with storage time (Sheftel, 2000). A small retail survey (47 samples) revealed <0.01 to 18.6 mg/kg DCHP in confectionery, snack products and biscuits wrapped in printed polypropylene film.

DCHP has also been identified in modeling clay (4000 mg/kg), pajamas (3400 mg/kg; TNO, 2003), and perfume (3 mg/kg in 1 of 36 perfume samples; SCCP, 2007).

## 7. DISCUSSION

Appendix A provides a summary of the NOAEL and LOAEL values for organ-specific endpoints for DCHP, all of which were derived from the multigeneration reproductive study by Hoshino et al. (2005) and the gestational exposure studies by Saillenfait et al. (2009) and Yamasaki et al. (2009). In these studies, the most sensitive developmental effects in pups were decreased AGD and increased nipple retention in male offspring. Prolonged preputial separation and testicular and kidney lesions in 10-week-old pups were also observed in one study. These responses are suggestive of an anti-androgen effect of DCHP in rats.

The multigeneration study by Hoshino et al. (2005) provided the most sensitive evidence of anti-androgen effects of DCHP, with significantly decreased AGD (7–9%) and increased incidence of areola mammae in male F1 and F2 offspring (16 and 63% in F1 and F2 males, respectively), at a LOAEL of 1,200 ppm in the diet (80–107 mg/kg-day). This study identified a NOAEL for developmental effects at 240 ppm (16–21 mg/kg-day). The gestational exposure studies found anti-androgen effects at higher dose levels (250–500 mg/kg-day by gavage) (Saillenfait et al., 2009; Yamasaki et al., 2009).

The Hoshino et al. (2005) study found subtle reproductive effects in F1 parental males at the same dose levels as the developmental effects. The LOAEL was 1,200 ppm in the diet (corresponding to a dose of 18 mg/kg-day in the affected group) and the NOAEL was 240 ppm in the diet (90 mg/kg-day). The observed effects were atrophy of seminiferous tubules (graded as severe in some high-dose males) and decreased spermatid head counts in F1 males. There were no effects on other sperm parameters in the F1 males and no effects on testes or sperm in F0 males. Reproductive success was not affected in any group. In other studies, testicular atrophy was reported in 1/5 male rats given 2,500 mg/kg-day by gavage for 7 days (Lake et al., 1982) and in male rats treated with 4,170 mg/kg-day by gavage for 21 days (Grasso, 1978, as cited in NICNAS, 2008).

Increased liver weight was the most sensitive endpoint in the parental animals in all of these studies, and occurred, relative to the anti-androgen effects in offspring, at lower doses in one gestational exposure study (100 mg/kg-day, Yamasaki et al., 2009), higher doses in the other gestational exposure study (500 mg/kg-day, Saillenfait et al., 2009), and the same doses in the multigeneration study (1,200 ppm or approximately 100 mg/kg-day, Hoshino et al., 2005). Investigation of hepatic endpoints was typically limited in these studies, but there were a few related observations, including induction of hepatic peroxisomal B oxidation in rats treated with DCHP on GDs 6–20 at all doses tested (250–750 mg/kg-day) and 1.5- and 2.2-fold increases in the activities of AST and ALT, respectively, at the high dose of 750 mg/kg-day (Saillenfait et al., 2009). In the two-generation study (Hoshino et al., 2005), significant increases in the incidence of hepatocellular hypertrophy were found in F0 and F1 adults at the high dose (6,000 ppm or 402–534 mg/kg-day).

Liver effects were also reported in other repeated-dose studies. These effects were best documented in the 7-day gavage study by Lake et al. (1982) that showed induction of hepatic enzymes, increased relative liver weight, proliferation of smooth endoplasmic reticulum, and hepatocellular hypertrophy occurred in rats administered DCHP at 1,500 mg/kg-day, with some

of these changes apparently occurring at doses down to 500 mg/kg-day. Liver effects unrelated to enzyme induction were reported in some of the other studies, including increased serum alkaline phosphatase and unspecified liver lesions (e.g., de Ryke and Bosland, 1978, as cited in European Commission, 2000; and de Ryke and Willems, 1977, as cited in European Commission, 2000 and NICNAS, 2008), but the documentation of these studies was poor and insufficient to evaluate the reported effects.

**Overall Uncertainty**

The animal hazard database for DCHP consisted of an acute repeat dose study, a multigeneration study, and two developmental studies, all of which were well described. A variety of other less well described animal studies were also performed. No human studies have been performed with DCHP.

**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-term and intermediate-term incidental oral exposures to DCHP were selected from a multigeneration reproductive study by Hoshino et al. (2005) and gestational exposure studies by Saillenfait et al. (2009) and Yamasaki et al. (2009). 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 DCHP induced changes in body weight (adult, maternal, fetus, pup), organ weight (liver, kidney, thyroid, brain), liver activity (maternal), and reproduction (post implantation loss, number pups born, mean estrous cycle, anogenital distance in fetus, F1 and F2 pups, homogenization resistant sperm). BMD software was also used for analysis of dichotomous data on DCHP-induced changes in development (incidence of cervical ribs), reproduction (incidence of litters with resorptions, hypospadias, testicular atrophy, areola with no nipple), and other organ pathologies (hypertrophic hepatocytes, kidney pathology, thyroid hypertrophy of follicular cells). 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 (body weight, liver 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, a low $BMDL_{10}$ value to high $BMDL_{10}$ value ratio of > 3. Following the screen, model selection preference was given to runs with high p-values, high Akaike's Information Criterion (AIC), and data points near estimated BMD and BMDL levels.

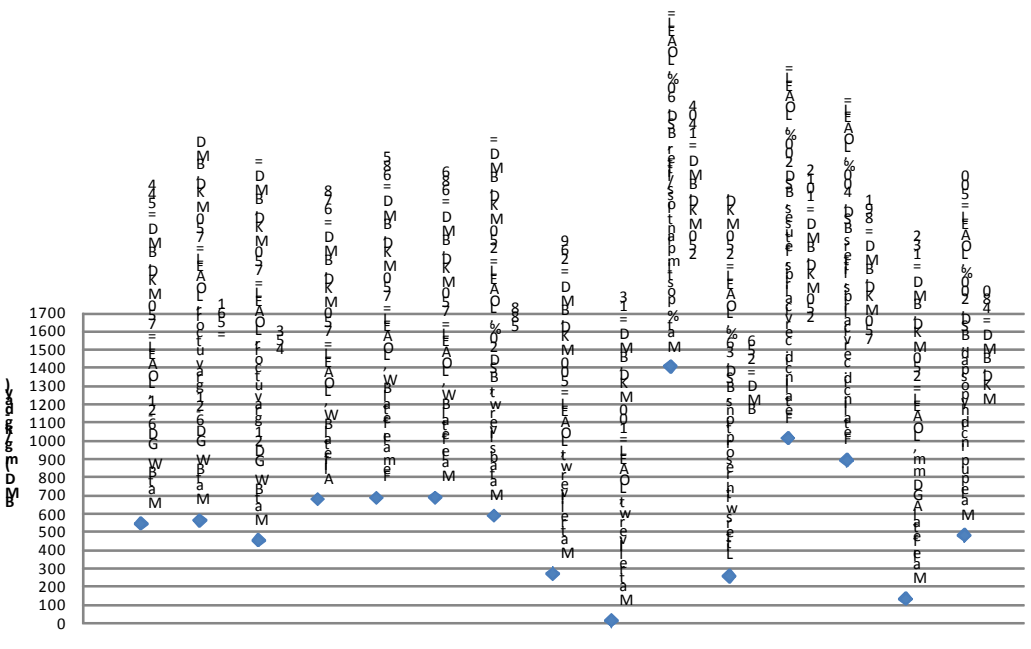
BMDS dichotomous models were selected to model data based on quantal variables. The $BMDL_{10}$ was also estimated for these data, but using different models (Gamma, Logistic, Multistage, Probit, and Weibull). For these endpoints, a 10% change was considered reasonable because of the severity of the adverse effect. This effect level is different than that used previously ($BMD_{05}$) by a Chronic Hazard Advisory Panel convened by CPSC (2001), 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 results of selected endpoints can be seen for gavage (Figure 7.1, 7.2) and dietary (Figure 7.3, 7.4) data. Summarized $BMD_{10}$ and $BMDL_{10}$ results and graphs can also be seen in Appendix C.

When looking at gavage dosing data, $BMDL_{10}$s range from 10 – 202 mg/kg-day for increases in liver weight, 299 – 561 mg/kg-day for body weight decrements, and 79 – 840 mg/kg-day for reproductive and developmental deficits.

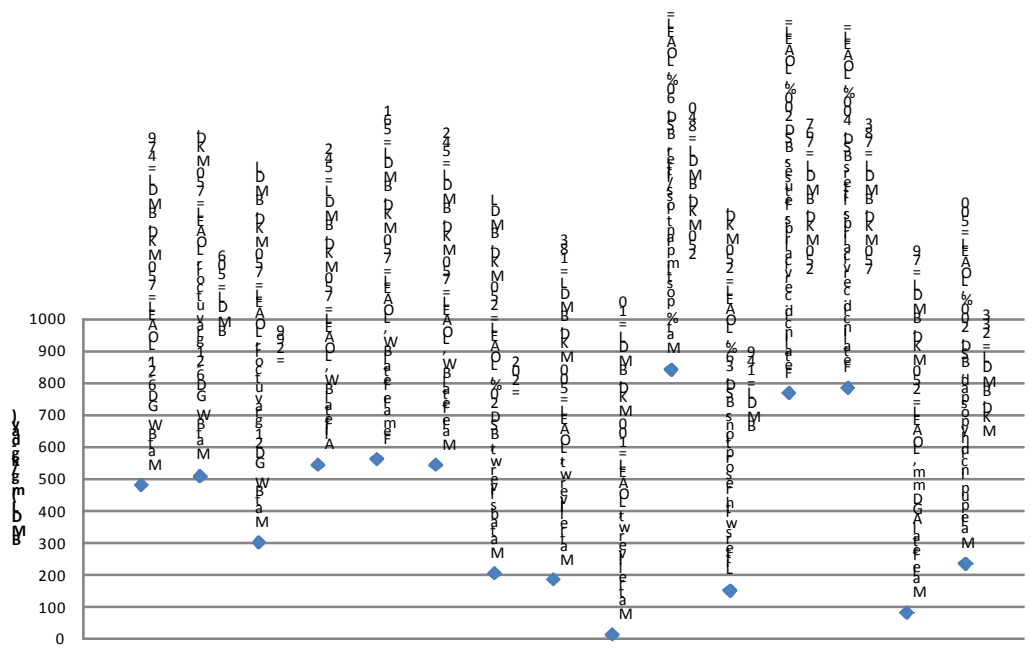
When looking at dietary dosing data, $BMDL_{10}$s range from 5.1 – 32 mg/kg-day for increases in liver weight, 41 - 73 mg/kg-day for body weight decrements, 45 – 91 mg/kg-day for reproductive and developmental deficits, and 32 – 190 mg/kg-day for other organ weight decrements and pathologies.

Figure 7.1 BMD Results for Toxicological Endpoints Induced by Gavage Dosing



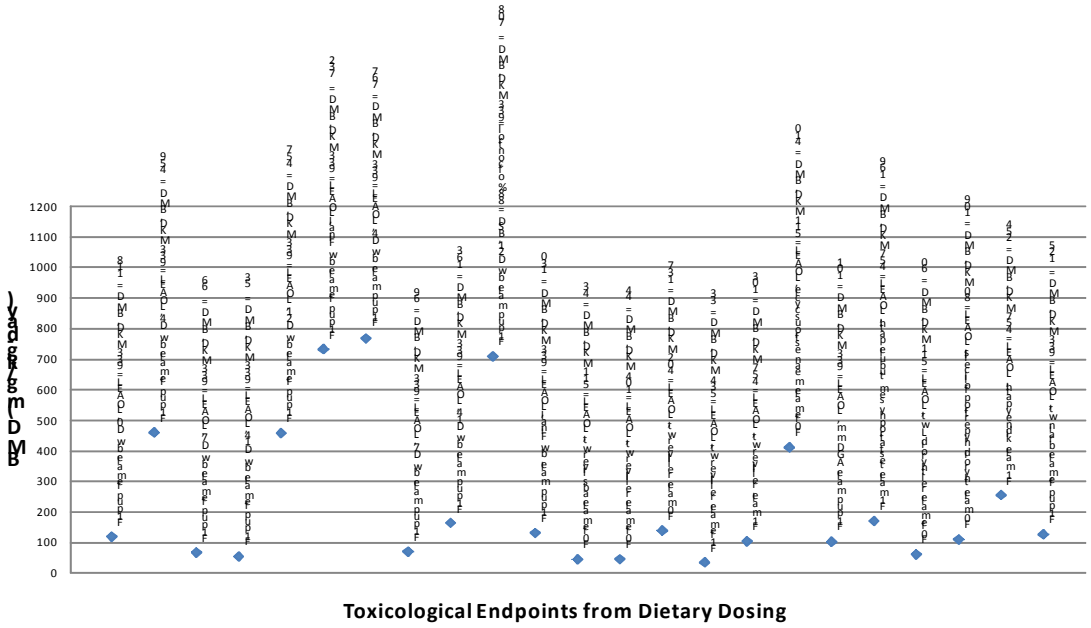

MKD = mg/kg-day; BSD = Biologically significant difference

Figure 7.2 BMDL Results for Toxicological Endpoints Induced by Gavage Dosing



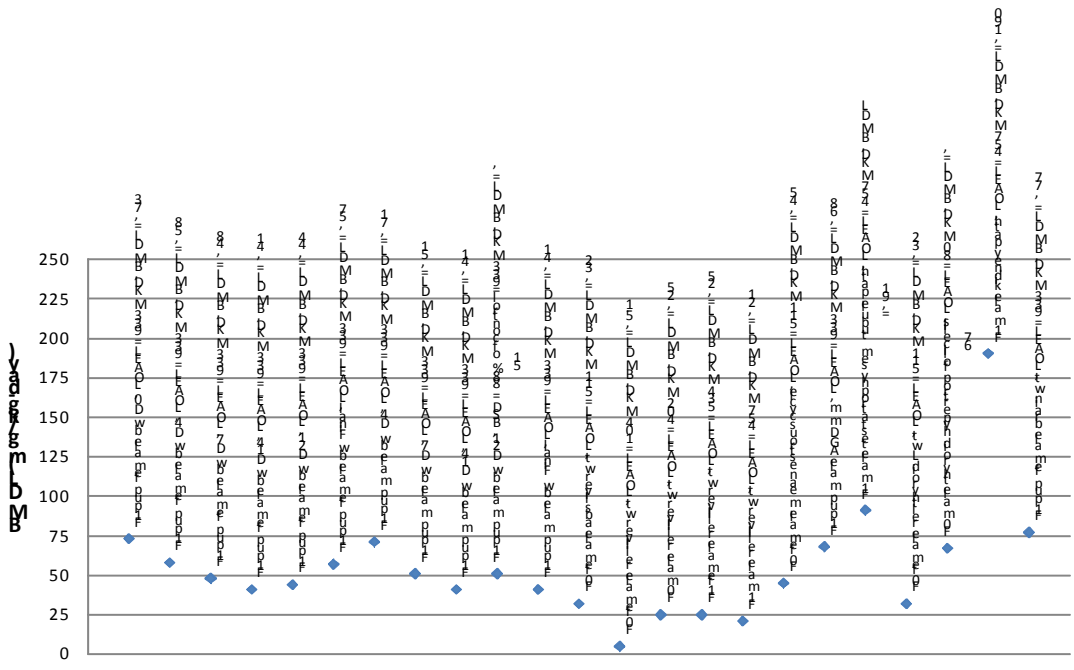

MKD = mg/kg-day; BSD = Biologically significant difference

Figure 7.3 **BMD Results for Toxicological Endpoints Induced by Dietary Dosing**




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

Figure 7.4 **BMDL Results for Toxicological Endpoints Induced by Dietary Dosing**




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

## Overall Acceptable Daily Intakes

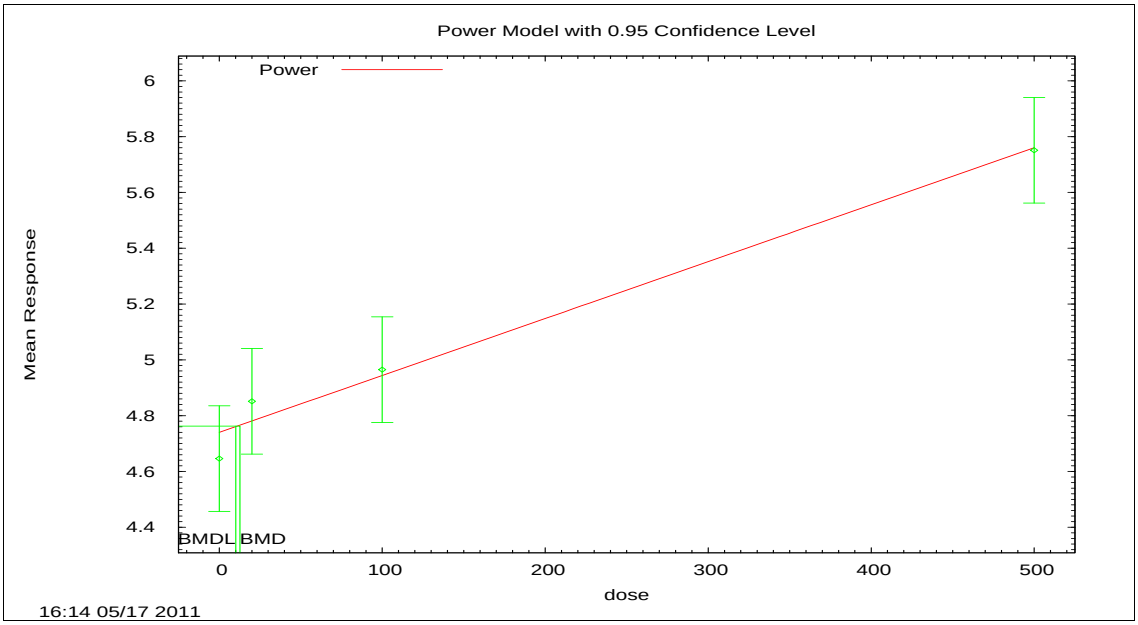
Acceptable daily intakes values (ADI's) are calculated when a given chemical is considered "toxic" and sufficient toxicity information is available. The ADI is the amount of a chemical that one may be exposed to on a daily basis without posing a significant risk of health effects to consumers. ADI's were estimated for short- and long-term exposure durations for the general population (non-reproductive endpoint) and long-term exposures to females (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 10 mg/kg-day (Yamasaki 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 male and female Sprague-Dawley rats were gavage dosed with DCHP in the feed during gestation day 6 to post-natal day 20. DCHP doses of 100 mg/kg-day (LOAEL) significantly increased the relative liver weight in the Sprague-Dawley dams. $BMDL_{10}$ model calculations suggested that the increase in F0 female relative liver weight was best described by the Power model (AIC = -62.3, model dependency ratio = 2.82 [< 3], goodness of fit p-value = 0.33; see Figure 7.5 below).

**Figure 7.5 Power Model Plot of Dam Relative Liver Weight (Yamasaki et al., 2009)**




Choice of liver study data for use as a hazard endpoint induced by short-term DCHP exposure was supported by additional liver and other organ data that had slightly higher hazard effect levels. Calculated $BMDL_{10}$s for changes in maternal absolute and relative liver weight were 202 and 183 mg/kg-day (LOAEL = 250 and 500 mg/kg-day), respectively. Maternal and fetal body weight changes had slightly higher $BMDL_{10}$s (range 299-561 mg/kg-day; LOAEL = 750 mg/kg-day; Saillenfait et al., 2009).

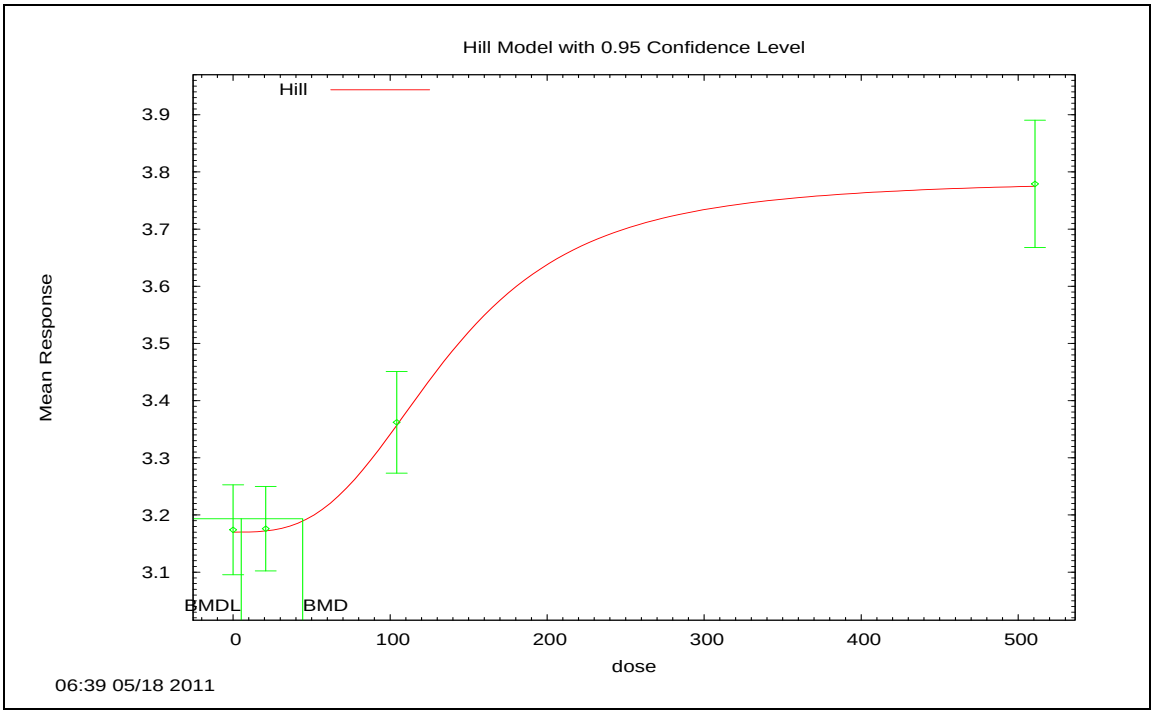
The $BMDL_{10}$ of 10 mg/kg-day was used to generate an Acceptable Daily Intake (ADI) by dividing by an uncertainty factor of 100 (10X for interspecies variation, 10X for intraspecies variation and sensitive populations). This "safety factor" is typically applied by CPSC to the lowest NO[A]EL for animal 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 0.1 mg/kg-day.**

***Long-term oral exposures – general population***

For long-duration oral exposures, the $BMDL_{10}$ of 5.1 mg/kg-day (Hoshino et al., 2005) was chosen as the representative overall hazard endpoint for general toxicity. This endpoint was derived from a reproduction study in which male and female Sprague-Dawley rats were dosed

with DCHP in the feed for 2 generations. DCHP doses of 104 mg/kg-day (LOAEL) increased the relative liver weight in these F0 female rats. $BMDL_{10}$ model calculations suggested that the increase in F0 female relative liver weight was best described by the Hill model (AIC = -184, model dependency ratio = 2.94 [< 3], goodness of fit p-value = unavailable; see Figure 7.6 below).

**Figure 7.6 Hill Model Plot of F0 Female Relative Liver Weight (Hoshino et al., 2005)**




Choice of liver study data for use as a hazard endpoint was supported by additional long-term liver and other organ data with slightly higher hazard effect levels. Increased absolute and relative liver weight have been reported in F0 male and female rats, and F1 male and female rats at LOAELs ranging from 402 – 534 mg/kg-day ($BMDL_{10}$s from 21 – 32 mg/kg-day; Hoshino et al., 2005). Changes in the F0 female relative left thyroid weight and histopathology, F1 female pup brain weight, and F1 male pup relative kidney pathology have also been reported at LOAELs = 80 – 933 mg/kg-day ($BMDL_{10}$s of 32 – 190 mg/kg-day).

The $BMDL_{10}$ of 5.1 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 0.051 mg/kg-da**y.

***Reproductive ADI***

***Long-term oral exposures – reproduction***

For long-duration oral exposures and reproductive endpoints, the $BMDL_{10}$ of 41 mg/kg-day (Hoshino et al., 2005) was chosen as the representative overall hazard endpoint for general toxicity. This endpoint was derived from a reproduction study in which male and female Sprague-Dawley rats were dosed with DCHP in the feed for 2 generations. DCHP doses of 933 mg/kg-day (LOAEL) decreased the body weight of F1 female pups (D14) and F1 male pups (D14, Final)

$BMDL_{10}$ model calculations suggested that the decrease in F1 female pup weight (D14), F1 male pup weight (D14), and F1 male pup weight (Final) were best described by the Linear (AIC = 236, model dependency ratio = 1.91 [< 3], goodness of fit p-value = 0.76), Exponential (AIC = 230, model dependency ratio = 1.63 [< 3], goodness of fit p-value = 0.8), and Exponential (AIC = 350, model dependency ratio = 1.91 [< 3], goodness of fit p-value = 1.0), models, respectively (see Figures 7.7a, b,c below).

**Figure 7.7a Linear Model Plot of F1 Female Pup Body Weight (D14; Hoshino et al., 2005)**

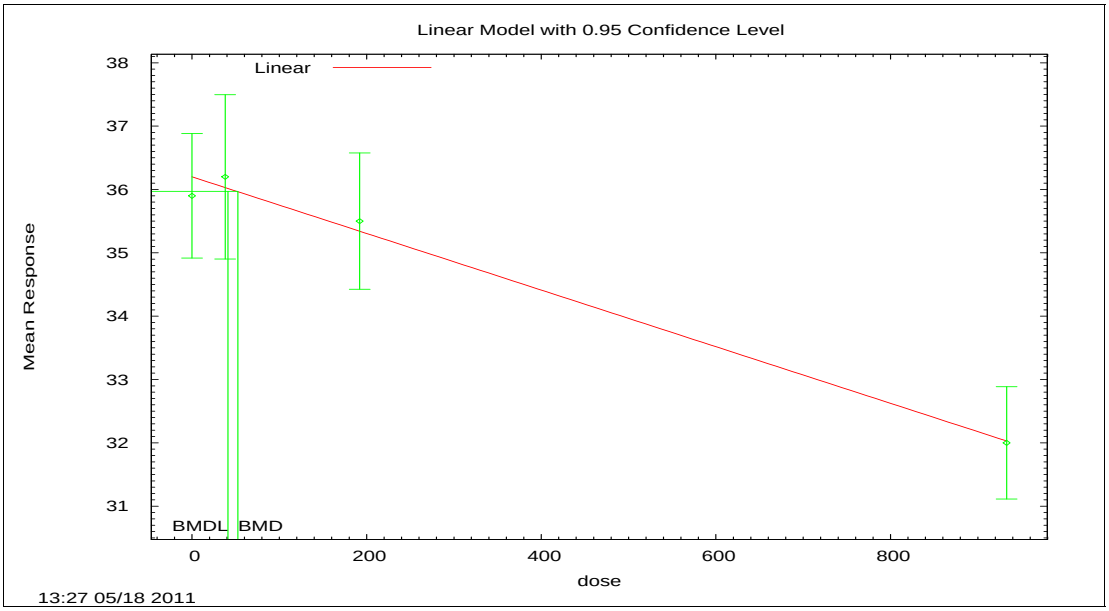



**Figure 7.7b Exponential Model Plot of F1 Male Pup Body Weight (D14; Hoshino et al., 2005)**

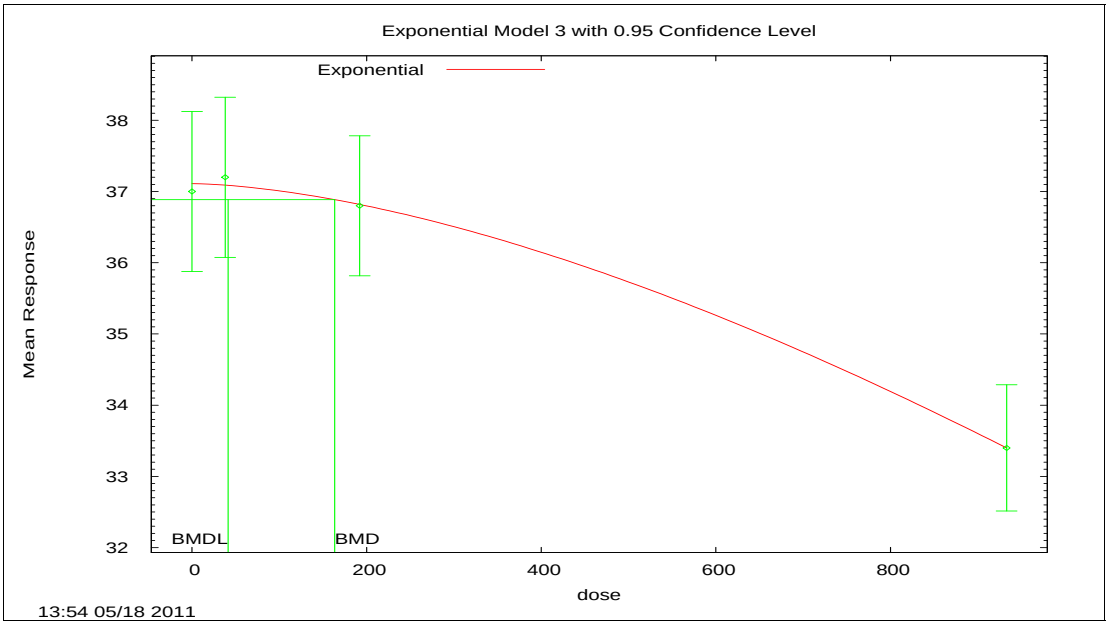
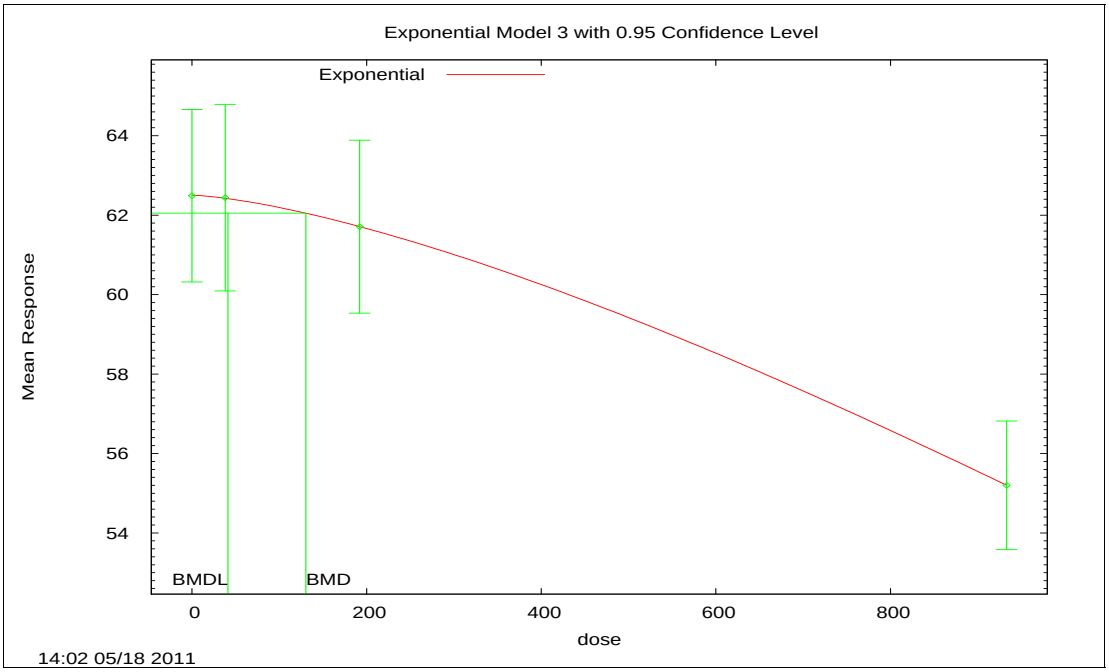


**Figure 7.7c Exponential Model Plot of F1 Male Pup Body Weight (Final; Hoshino et al., 2005)**




Choice of male and female body weight data was supported by additional reproduction-related data with slightly higher hazard effect levels. Decreased F1 male (D4, D7, D21) and female pup (D0, D4, D7, D21, Final) body weights were reported at a LOAEL of 933 mg/kg-day ($BMDL_{10}$s = 44 – 73 mg/kg-day). Increased female mean estrous cycle was observed at a LOAEL of 511 mg/kg-day ($BMDL_{10}$ = 45 mg/kg-day) and increased incidence of F1 male testicular atrophy and seminiferous tubule pathology was revealed at a LOAEL = 457 mg/kg-day ($BMDL_{10}$ = 91 mg/kg-day).

The $BMDL_{10}$ of 41 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 0.41 mg/kg-day.**

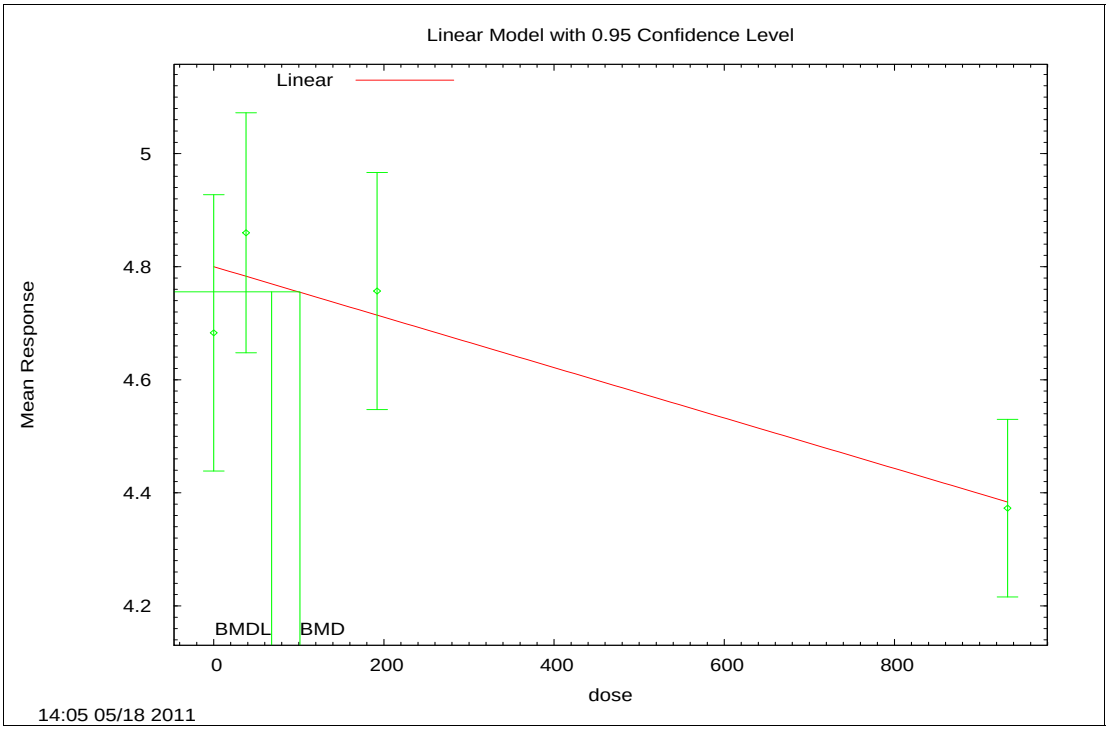
***Developmental ADI***

***Maternal exposures – developmental effects***

For developmental effects, the maternal dose $BMDL_{10}$ of 68 mg/kg-day was chosen as the representative overall hazard endpoint (Hoshino et al., 2005)**.** This endpoint was derived from a reproduction study in which male and female Sprague-Dawley rats were dosed with DCHP in the feed for 2 generations. DCHP doses of 933 mg/kg-day (LOAEL) decreased the anogenital distance in F1 male rat pups. $BMDL_{10}$ model calculations suggested that the decrease in F1 male rat pup anogenital distance was best described by the Linear model (AIC = -44, model dependency ratio = 2.12 [< 3], goodness of fit p-value = 0.33; see Figure 7.8 below).

The $BMDL_{10}$ was higher than those that induced systemic toxicity (liver, thyroid, kidney) in F0 dams. Decreases in ano-genital distance are not characteristic of general systemic toxicity, however, but are a characteristic effect of phthalates that induce developmental effects. The choice of this effect as a hazard endpoint is therefore warrented.

**Figure 7.8 Hill Model Plot of F1 Male Pup Anogenital Distance (Hoshino et al., 2005)**




Choice of the developmental study data for use as a hazard endpoint was supported by additional studies with slightly higher hazard effect levels. The $BMDL_{10}$ calculated for decreases in anogenital distance in another study was 79 mg/kg-day (LOAEL = 250 mg/kg-day; Saillenfait et al., 2009). Increased incidence of hypospadias (also a hallmark effect of phthalates that induce

developmental effects) occurred at a $BMDL_{10}$ of 233 mg/kg-day (LOAEL = 500 mg/kg-day). Increased incidence of fetal cervical ribs occurred at 767 (per fetus basis; LOAEL = 250 mg/kg-day) and 783 (per litter basis; LOAEL = 750 mg/kg-day) mg/kg-day.

The $BMDL_{10}$ of 68 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 0.68 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 DCHP by Organ System**

| Species (Gender) | Exposure Route | Dose (Number of Animals per Dose Group) | Dose Duration | Effect Category | Toxicological Endpoint | Toxicological Basis | Citation |
|---|---|---|---|---|---|---|---|
| Sprague-Dawley rat (M&F) | Oral (diet) | 0, 240, 1,200, or 6,000 ppm<br><br>0, 16–21, 80–107, or 402–534 mg/kg-day (calculated by the researchers) (24/group) | During pre-mating, mating, gestation, and lactation for two generations | General | NOAEL = 18–21 mg/kg<br>LOAEL = 90–104 mg/kg | Decreased body weight (F0 females and F1 males most sensitive) | Hoshino et al., 2005 |
| | | | | Liver | NOAEL = 21 mg/kg-day<br>LOAEL = 104 mg/kg-day | Increased liver weight (F0 females most sensitive) | |
| | | | | Kidney | NOAEL = 80–90 mg/kg-day<br>LOAEL = 402–457 mg/kg-day | Increased severity of hyaline droplets in the renal proximal tubular epithelium (F0 and F1 males) | |
| | | | | Endocrine | NOAEL = 80–107 mg/kg-day<br>LOAEL = 402–534 mg/kg-day | Hypertrophy of thyroid follicular epithelial cells and increased thyroid weight (F0 and F1 males and females) | |
| | | | | Reproduction | Parental males:<br>NOAEL = 18 mg/kg-day<br>LOAEL = 90 mg/kg-day<br>Parental females:<br>NOAEL = 511–534 mg/kg-day<br>LOAEL = None | Seminiferous tubule atrophy and decreased spermatid head counts in F1 males; no toxicologically significant effects in parental females; no effects on male or female reproductive success | |
| | | | | Development/fetus | NOAEL = 16–21 mg/kg-day<br>LOAEL = 80–107 mg/kg-day | Decreased AGD and increased incidence of areola mammae in male F1 and F2 offspring | |

| Table A.1. Summary of NOAELs/LOAELs Identified for DCHP by Organ System | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Species (Gender)** | **Exposure Route** | **Dose (Number of Animals per Dose Group)** | **Dose Duration** | **Effect Category** | **Toxicological Endpoint** | **Toxicological Basis** | **Citation** |
| Sprague-Dawley rat (F) | Gavage in olive oil | 0, 250, 500, or 750 mg/kg-day (24–25/group; 6–9/group for liver endpoints) | GDs 6–20 (sacrificed on GD 21) | General | NOAEL = 250 mg/kg-day<br>LOAEL = 500 mg/kg-day | Decreased body weight gain | Saillenfait et al., 2009 |
| | | | | Liver | NOAEL = 250 mg/kg-day<br>LOAEL = 500 mg/kg-day | Increased relative liver weight | |
| | | | | Development/Fetus | NOAEL = None<br>LOAEL = 250 mg/kg-day | Decreased AGD in males | |
| Sprague-Dawley rat (F) | Gavage in olive oil | 0, 20, 100, or 500 mg/kg-day (10/group) | GDs 6–20 (allowed to give birth) | General | NOAEL = 500 mg/kg-day<br>LOAEL = None | No significant change in body weight | Yamasaki et al., 2009 |
| | | | | Liver | NOAEL = 20 mg/kg-day<br>LOAEL = 100 mg/kg-day | Increased relative liver weight | |
| | | | | Development/fetus | NOAEL = 100 mg/kg-day<br>LOAEL = 500 mg/kg-day | Slightly decreased pup viability on PND 4; decreased pup body weight on PND 14/21; decreased AGD, increased areola/nipple retention, prolonged preputial separation, and slight histological changes in testis and kidney in male pups | |

## Appendix B. Critical Study Reviews

Lake et al. (1982)

Male Sprague-Dawley rats (5–12/group) were administered DCHP (≥99% pure) in corn oil via gavage at 0, 500, 1,000, 1,500, 2,000, or 2,500 mg/kg-day for 7 days (Lake et al., 1982). At study termination, animals were fasted overnight and sacrificed. Livers, kidneys, and testes were excised, weighed (all dose groups), and subjected to biochemical (all dose groups) and/or histopathological (0, 1,500, and 2,500 mg/kg-day groups only) analyses. Ultrastructural examination was performed on the liver of 0 and 1,500 mg/kg-day animals.

Body weights and absolute organ weights were not reported. A dose-related increase in relative liver weight was observed in DCHP-treated rats; relative liver weight was increased by 42% in rats treated with 1,500 mg/kg-day DCHP compared to vehicle-only controls (Table B.1). Numerical data for other dose groups were not provided in the original study report. No changes in relative kidney or testes weights were seen in treated rats compared to controls.

**Table B.1. Significant Effects in Male Sprague-Dawley Rats Administered DCHP in Corn Oil for 7 Days**

| | Dose (mg/kg-day) | |
|---|---|---|
| | **0** | **1,500** |
| Relative liver weight (g/100 g body weight) | 3.3 ± 0.1[a] | 4.7 ± 0.1 (142)[b,c] |
| Hepatic enzyme levels | | |
| 7-Ethoxycoumarin-0-deethylase (µmol/hour/g liver) | 5.8 ± 0.4 | 16.7 ± 1.3 (288)[c] |
| Biphenyl 4-hydroxylase (µmol/hour/g liver) | 8.7 ± 0.6 | 20.4 ± 1.3 (234)[c] |
| Aniline 4-hydroxylase (µmol/hour/g liver) | 4.3 ± 0.3 | 5.5 ± 0.3 (128)[d] |
| Cytochrome P450 (nmol/mg microsomal protein) | 1.04 ± 0.04 | 1.45 ± 0.02 (139)[c] |
| Cytochrome $b_5$ (nmol/mg microsomal protein) | 0.40 ± 0.02 | 0.47 ± 0.02 (118)[d] |
| Microsomal heme (nmol/mg microsomal protein) | 1.53 ± 0.05 | 1.91 ± 0.04 (125)[c] |

[a]Mean ± SEM for groups of 6–12 animals.
[b]Percentage of control values shown in parentheses.
[c]Significantly different from controls at $p < 0.001$.
[d]Significantly different from controls at $p < 0.05$.

Source: Lake et al. (1982).

Significant induction of several enzymes, including 7-ethoxycoumarin 0-deethylase (288% of control), biphenyl 4-hydroxylase (234% of control), and aniline 4-hydroxylase (128% of control), and increased levels of microsomal cytochrome P450 (139% of control), cytochrome

$b_5$ (118% of control), and heme (125% of control) were seen in the livers of rats treated with DCHP at 1,500 mg/kg-day (Table B.1). Again, numerical data for other dose groups were not provided in the original study report. Based on data presented graphically, relative liver weight, 7-ethoxycoumarin 0-deethylase activity, and hepatic cytochrome P450 content appeared to differ substantially from controls at doses <1,500 mg/kg-day (and as low as 500 mg/kg-day). However, statistical analyses were not performed.

Slight hypertrophy of centrilobular cells of the liver was noted at 1,500 mg/kg-day; this effect was more marked in rats dosed with 2,500 mg/kg-day DCHP. Ultrastructural analyses showed that treatment with 1,500 mg/kg-day DCHP led to marked proliferation of the smooth endoplasmic reticulum of the centrilobular hepatocytes. Other intracellular organelles looked similar to controls, and there was no evidence of peroxisome proliferation. No lesions were detected in the kidneys. One of five animals treated with 2,500 mg/kg-day DCHP exhibited bilateral tubular atrophy of 30–40% of the germinal cells of the testes. None of the other examined animals showed any abnormalities in the testes.

In summary, treatment with DCHP elicited hepatic enzyme induction, increased relative liver weight, proliferation of smooth endoplasmic reticulum, and hepatocellular hypertrophy at 1,500 mg/kg-day in this study, with some of these effects apparently occurring at doses as low as 500 mg/kg-day.

Hoshino et al. (2005)

The reproductive and prenatal, perinatal, and postnatal toxicity of DCHP was investigated by Hoshino et al. (2005). Doses of DCHP for the two-generation reproductive/developmental toxicity study were selected based on the results of a preliminary toxicity study. In the preliminary dose range-finding study, Sprague-Dawley rats (group sizes not reported) were administered DCHP (99.9% pure) in the diet at 0, 600, 2,000, 6,000, or 20,000 ppm during the period from 3 weeks or more before mating, through mating, and until necropsy (males) or through gestation and lactation periods (females) (Hoshino et al., 2005). Parameters evaluated were not explicitly specified. However, the researchers reported inhibition of body weight gain and increased liver weights in rats administered the high dose; high-dose females also exhibited increased adrenal weights and decreased thymus, spleen, and ovary weights (data not shown; absolute or relative weights not specified). There were no effects on reproductive function, delivery, or lactation; the only effect reported in the offspring of treated rats was inhibition of body weight gain (6,000 and 20,000 ppm treatment groups).

Based on these data, the main (two-generation) study design consisted of groups of 24 male and female Sprague-Dawley rats that were administered DCHP (99.9% pure) in the diet at 0, 240, 1,200, or 6,000 ppm (Hoshino et al., 2005). Dosing of F0 parents was initiated at 5 weeks of age and continued through ≥10 weeks of pre-mating and mating (males) or ≥10 weeks of pre-mating, mating, gestation, and lactation periods (until weaning of F1 offspring on PND 21; females). F1 animals were administered DCHP starting from the time of weaning (PND 21) and through the same periods of pre-mating, mating, gestation, and lactation. F0 and F1 animals that failed to deliver a litter were administered DCHP until sacrifice (at least 26 days after copulation). Daily chemical intake averaged over the total study period for each of the dosage groups (calculated by the researchers) is shown in Table B.2. Parameters evaluated in parental animals included mortality and clinical signs of toxicity (daily), body weights and food consumption (weekly, and on GDs 0, 7, 14, and 20 and lactation days 0, 4, 7, 14, and 21), estrous cycle length (from vaginal smears acquired 2 weeks leading up to mating), sperm parameters (such as number and motility, homogenization-resistant spermatids and abnormal sperm), hormone levels (testosterone, follicle-stimulating hormone [FSH], and luteinizing hormone [LH] in males and FSH and LH in females), organ weights (14 organs), and comprehensive histopathological analyses. Endpoints evaluated in pups included clinical signs of toxicity (through lactation), body weights (PNDs 0, 4, 7, 14, and 21), measurement of AGD (on PND 4), physical development and sexual maturation (including appearance of areola, incidence of pinna unfolding, timing of upper incisor eruption and eye opening, preputial separation, and vaginal opening), reflex response, organ weights (brain, thymus, and spleen), and pathological examinations (all pups). The litter was considered the unit for statistical analyses.

**Table B.2. Daily Chemical Intake for Sprague-Dawley Rats Administered DCHP in the Diet in a Two-Generation Study**

| Group | Dietary concentration (ppm) | | | |
|---|---|---|---|---|
| | **0** | **240** | **1,200** | **6,000** |
| Dose (mg/kg-d) | | | | |
| F0 males | – | 15.88 | 79.57 | 401.8 |
| F0 females | – | 20.80 | 104.19 | 510.7 |
| F1 males | – | 17.84 | 89.89 | 457.4 |
| F1 females | – | 20.95 | 107.15 | 534.2 |

Source: Hoshino et al. (2005).

Significant effects in parental animals associated with DCHP treatment are summarized in Table B.3. Parental animals (F0 females and F1 males and females) showed significant inhibition of body weight gain; final body weights of F0 females were 5–8% lower and F1 males

were 10–12% lower at the two highest doses than concurrent controls (Hoshino et al., 2005). Food consumption was likewise reduced in the same parental groups. Compared to controls, spermatid head counts in the testes were decreased in mid- and high-dose F1 males by 15 and 24%, respectively. Other sperm parameters were unaffected. In mid- and high-dose animals, hypertrophy of hepatocytes and thyroid follicular epithelial cells (F0 and F1 males and females) and atrophy of seminiferous tubules (F1 males) were also observed; the incidences of these lesions were significantly increased at 6,000 ppm compared to controls. Liver and thyroid weights were increased in parental rats; the increases were statistically significant primarily at the high dose, but also for relative liver weight in F0 females at 1,200 ppm. Absolute prostate weights were reduced in F1 males at all doses relative to controls, but relative prostate weight was decreased only at the high dose. Prostate weights were unchanged from controls in the F0 males. Additional effects seen at the high-dose included a slight increase in estrous cycle length (F0 females) and increased severity of hyaline droplets in the renal proximal tubular epithelium (F0 and F1 males). No effects were observed on serum hormone levels or reproductive capability (mating, fertility, gestation, and birth index).

**Table B.3. Significant Parental Effects in Sprague-Dawley Rats Administered DCHP in the Diet in a Two-Generation Study**

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 240 | 1,200 | 6,000 |
| **Parental animals** | | | | |
| Body weight at study termination (g) | | | | |
| F0 males | 510.5 ± 50.4[a] | 503.6 ± 42.5 | 500.4 ± 28.0 | 496.7 ± 37.1 |
| F0 females | 322.1 ± 19.7 | 311.9 ± 16.6 | 306.5 ± 18.2[b] | 295.3 ± 18.8[c] |
| F1 males | 624.9 ± 48.9 | 603.4 ± 54.0 | 564.7 ± 42.0[c] | 552.5 ± 30.5[c] |
| F1 females | 337.4 ± 27.8 | 338.7 ± 23.9 | 330.8 ± 18.8 | 320.7 ± 22.7 |
| Estrous cycle length (days) | | | | |
| F0 females | 4.04 ± 0.14 | 4.06 ± 0.22 | 4.00 ± 0.00 | 4.25 ± 0.42[b] |
| Spermatid head count ($10^6$/g) | | | | |
| F1 males | 104.0 ± 12.66 | 93.4 ± 10.27 | 88.6 ± 10.32 [b] | 79.2 ± 30.29[c] |
| Organ weights; F0 adults | | | | |
| Absolute thyroid weight (left) (mg) | 11.33 ± 2.65[d]<br>10.08 ± 2.15[e] | 12.48 ± 2.47<br>9.74 ± 1.59 | 11.99 ± 2.11<br>9.91 ± 1.80 | 14.37 ± 4.02[c]<br>11.35 ± 1.39 |
| Relative thyroid weight (left) (percent body weight × 1,000) | 2.23 ± 0.51[d]<br>3.13 ± 0.68[e] | 2.48 ± 0.46<br>3.12 ± 0.49 | 2.40 ± 0.43<br>3.23 ± 0.57 | 2.91 ± 0.91[c]<br>3.87 ± 0.54[c] |
| Absolute liver weight (g) | 15.042 ± 1.911[d]<br>10.233 ± 0.944[e] | 14.620 ± 1.960<br>9.907 ± 0.772 | 14.596 ± 1.183<br>10.298 ± 0.824 | 18.157 ± 1.730[c]<br>11.157 ± 0.995[c] |
| Relative liver weight (percent body weight) | 2.944 ± 0.203[d]<br>3.174 ± 0.168[e] | 2.898 ± 0.229<br>3.176 ± 0.171 | 2.917 ± 0.177<br>3.362 ± 0.190[b] | 3.658 ± 0.252[c]<br>3.779 ± 0.251[c] |
| Organ weights; F1 adults | | | | |

| Table B.3. Significant Parental Effects in Sprague-Dawley Rats Administered DCHP in the Diet in a Two-Generation Study | | | | |
|---|---|---|---|---|
| | Dose (ppm) | | | |
| **Parameter** | **0** | **240** | **1,200** | **6,000** |
| Relative liver weight (percent body weight) | 3.00 ± 0.210[d]<br>3.78 ± 0.455[e] | 2.88 ± 0.195<br>3.64 ± 0.360 | 2.94 ± 0.238<br>3.90 ± 0.467 | 3.42 ± 0.314[c]<br>4.39 ± 0.393[c] |
| Absolute prostate weight (g) | 0.71 ± 0.152[d] | 0.58 ± 0.133[c] | 0.59 ± 0.149[b] | 0.51 ± 0.118[c] |
| Relative prostate weight (percent body weight) | 0.11 ± 0.027[d] | 0.10 ± 0.20 | 0.11 ± 0.029 | 0.09 ± 0.024[b] |
| Histopathology; F0 adults | | | | |
| Liver: hypertrophy, hepatocytes, diffuse | 0/24[d]<br>0/24[e] | 0/24<br>0/24 | 4/24<br>3/24 | 16/24[f]<br>12/24[f] |
| Thyroid: hypertrophy, follicular cells | 0/24[d]<br>0/24[e] | 0/24<br>0/24 | 3/24<br>0/24 | 7/24[f]<br>6/24[f] |
| Kidney: hyaline droplet, proximal tubular epithelium; slight | 23/24[d]<br>0/24[e] | 22/24<br>NE | 23/24<br>0/1 | 9/24<br>0/24 |
| Kidney: hyaline droplet, proximal tubular epithelium; moderate | 1/24[d]<br>0/24[e] | 1/24<br>NE | 0/24<br>0/1 | 15/24[f]<br>0/24 |
| Histopathology; F1 adults | | | | |
| Liver: hypertrophy, hepatocytes, diffuse | 0/20[d]<br>0/20[e] | 0/23<br>0/23 | 0/20<br>0/20 | 14/22[f]<br>9/22[f] |
| Thyroid: hypertrophy, follicular cells | 0/20[d]<br>0/20[e] | 0/23<br>0/23 | 0/20<br>0/20 | 7/22[f]<br>6/22[f] |
| Kidney: hyaline droplet, proximal tubular epithelium; slight | 19/20[d]<br>0/20[e] | 22/23<br>NE | 20/20<br>NE | 14/22<br>0/22 |
| Kidney: hyaline droplet, proximal tubular epithelium; moderate | 1/20[d]<br>0/20[e] | 1/23<br>NE | 0/20<br>NE | 8/22[f]<br>0/22 |
| Testis: atrophy, seminiferous tubules | 1/20[d] | 0/23 | 2/20 | 9/22[f, g] |

[a]Mean ± SD.
[b]Significantly different from controls at $p < 0.05$ (as reported by the study authors).
[c]Significantly different from controls at $p < 0.01$.
[d]Males.
[e]Females.
[f]$p < 0.05$ compared to control group using Fisher's exact test (performed for this evaluation).
[g]Graded as severe in three cases.

NE = not examined

Source: Hoshino et al. (2005).

Significant effects observed in the offspring included inhibition of body weight gain (high-dose F1 and F2 litters; 4–12%), decreased AGD in males (by 7–9%; high-dose F1 pups and mid- and high-dose F2 pups), and increased incidence of areola mammae in males (high-dose F1 and mid- and high-dose F2 pups) (Table B.4; Hoshino et al., 2005). No effects with regard to other developmental milestones, clinical signs, number of pups delivered, sex ratio of

pups, pup viability, reflex and response tests, external abnormalities, or histopathology were observed.

**Table B.4. Significant Developmental Effects in Sprague-Dawley Rats Administered DCHP in the Diet in a Two-Generation Study**

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 240 | 1,200 | 6,000 |
| **Litter data** | | | | |
| Body weights; F1 offspring (g) | | | | |
| Day 0 | 6.8 ± 0.6[a,b]<br>6.5 ± 0.5[c] | 6.8 ± 0.5<br>6.4 ± 0.5 | 6.8 ± 0.4<br>6.4 ± 0.5 | 6.5 ± 0.4[d]<br>6.1 ± 0.4[d] |
| Day 21 | 62.2 ± 4.5[b]<br>59.2 ± 3.7[c] | 61.9 ± 4.8<br>59.6 ± 4.9 | 62.6 ± 4.6<br>59.4 ± 4.7 | 55.0 ± 3.8[e]<br>52.8 ± 3.2[e] |
| Final | 62.49 ± 4.64[b]<br>58.86 ± 3.93[c] | 62.44 ± 5.43<br>59.55 ± 5.39 | 61.71 ± 4.65<br>59.78 ± 5.17 | 55.20 ± 3.65[e]<br>52.27 ± 3.07[e] |
| Body weights; F2 offspring (g) | | | | |
| Day 0 | 6.8 ± 0.4[b]<br>6.4 ± 0.4[c] | 6.6 ± 0.5<br>6.2 ± 0.4 | 6.5 ± 0.5<br>6.2 ± 0.5 | 6.6 ± 0.6<br>6.2 ± 0.6 |
| Day 21 | 64.9 ± 4.2[b]<br>61.7 ± 3.7[c] | 62.8 ± 4.2<br>59.3 ± 3.5 | 62.8 ± 5.0<br>59.2 ± 4.4 | 59.2 ± 5.0[e]<br>56.6 ± 4.3[e] |
| Final | 66.36 ± 3.86[b]<br>60.59 ± 4.94[c] | 62.75 ± 5.30<br>59.70 ± 4.16 | 62.79 ± 6.23<br>59.11 ± 4.67 | 59.93 ± 6.48[e]<br>56.38 ± 4.66[e] |
| Physical development; male F1 offspring | | | | |
| AGD (mm) | 4.68 ± 0.522 | 4.86 ± 0.491 | 4.76 ± 0.448 | 4.37 ± 0.354[e] |
| AGD/body weight$^{1/3}$ | 2.17 ± 0.216 | 2.16 ± 0.213 | 2.11 ± 0.148 | 2.00 ± 0.151[d] |
| Incidence of areole mammae (percent) | 0.0 | 0.0 | 0.0 | 16.1[e] |
| Body weight at preputial separation (g) | 225.3 ± 17.3 | 225.1 ± 12.5 | 218.9 ± 15.4 | 212.5 ± 13.8[d] |
| Physical development; male F2 offspring | | | | |
| AGD (mm) | 4.62 ± 0.314 | 4.49 ± 0.300 | 4.28 ± 0.365[e] | 4.19 ± 0.387[e] |
| AGD/body weight$^{1/3}$ | 2.07 ± 0.152 | 2.02 ± 0.125 | 1.93 ± 0.158[e] | 1.88 ± 0.129[e] |
| Incidence of areola mammae (percent) | 0.0 | 0.0 | 18.4 | 63.2[e] |

[a]Mean ± SD.
[b]Males.
[c]Females.
[d]Significantly different from controls at $p < 0.05$.
[e]Significantly different from controls at $p < 0.01$.

Source: Hoshino et al. (2005).

Using these data, parental NOAEL and LOAEL values of 240 and 1,200 ppm are identified, based on decreased body weight and increased relative liver weight in F0 females (21 and 104 mg/kg-day, respectively) and decreased body weight in F1 males (18 and 90 mg/kg-day). NOAEL and LOAEL values of 240 and 1,200 ppm are also identified for reproductive toxicity based on significantly decreased spermatid head counts and seminiferous tubule atrophy

in F1 males (18 and 90 mg/kg-day) and for developmental toxicity based on decreased AGD and increased incidence of areola mammae in male F1 and F2 offspring (16–21 and 80–107 mg/kg-day).

Saillenfait et al. (2009)

Time-mated pregnant Sprague-Dawley rats (24–25 group) were administered DCHP (>99% pure) in olive oil by gavage at 0, 250, 500, or 750 mg/kg-day on GDs 6–20 and sacrificed on GD 21 (Saillenfait et al., 2009). Initial dosing formulations were based on body weights on GD 6; subsequent doses were adjusted based on body weight measurements recorded every 3 days throughout the treatment period. Animals were monitored daily for mortality and clinical signs of toxicity. Food consumption was measured every 3 days starting on GD 6. Maternal body weights were recorded on GDs 0, 6, 9, 12, 15, 18, and 21 (prior to sacrifice). The uteri of dams sacrificed on GD 21 were weighed; uterine contents were examined to determine numbers of implantation sites, resorptions, and dead and live fetuses. Uteri without implantation sites were evaluated (by staining with ammonium sulfide) to detect early resorptions. The number of corpora lutea in each ovary was recorded. Live fetuses were weighed, sexed, and examined for external anomalies (including those in the oral cavity). AGDs were measured using a dissecting microscope. Half of the live fetuses from each litter were examined for internal soft tissue changes; the other half was subjected to skeletal examinations. The sex of all fetuses was determined by internal examination of the gonads. The degree of TTM was determined by measuring (microscopically) the distance from the bladder neck to the lower pole of the testes. Since the liver is a potential target organ for toxicity (in dams), a satellite experiment was concurrently conducted to evaluate hepatotoxicity. In the satellite study, timed-pregnant rats (6–9/group) were administered DCHP under the same experimental conditions as the main study. At sacrifice on GD 21, liver weights were recorded; the left lobes were subjected to histological examination. The remaining liver samples were used to assess cyanide-insensitive palmitoyl CoA oxidase activity, which is a specific peroxisomal enzyme marker. Cholesterol, triglycerides, and the activities of AST and ALT were measured in the serum.

Significant effects in DCHP-treated rats are summarized in Table B.5. No mortality or clinical signs of toxicity were observed. However, rats treated with DCHP at the high doses of 750 mg/kg-day showed significant decreases in body weight gain (22%), body weight (12%), and food consumption relative to controls (Table B.5). Rats in the mid-dose group also experienced transitory decreases in food consumption and body weight gain early in gestation. No effects on the numbers of implantations or live fetuses, incidence of post-implantational loss

and resorption, or sex ratio of pups were observed. The body weights of male and female pups were decreased in a dose-related manner; an average weight reduction of 11% compared to controls was observed at 750 mg/kg-day. A significant and dose-related decrease in AGD was seen in all treated males; with respect to controls, AGD was decreased by 9, 12, and 17% in males treated at 250, 500, and 750 mg/kg-day, respectively. The incidences of external, soft tissue, and skeletal malformations and variations in treated rats were comparable to controls. Neither undescended testes nor TTM changes were observed in treated rats. The satellite study showed that relative, but not absolute, liver weights of dams were significantly higher than those of untreated controls at 500 and 750 mg/kg-day (17 and 28% higher, respectively). The activities of ALT and AST were elevated only in high-dose rats; mild but significant (1.7–2.1-fold) induction of hepatic peroxisomal B oxidation activity was observed in all treatment groups. No histopathological changes attributable to DCHP treatment were observed. Based on these data, parental NOAEL and LOAEL values of 250 and 500 mg/kg-day (for decreased body weight gain and increased relative liver weight) are identified. A LOAEL of 250 mg/kg-day (and no NOAEL value) is identified for developmental toxicity based on decreased AGD in male fetuses.

**Table B.5. Significant Changes in Sprague-Dawley Rats Administered DCHP via Gavage on GDs 6–20**

| Parameter | Dose (mg/kg-day) | | | |
|---|---|---|---|---|
| | **0** | **250** | **500** | **750** |
| **Parental animals** | | | | |
| Body weight change (g) | | | | |
| GDs 6–9 | 15 ± 5[a] | 13 ± 5 | 10 ± 5[b] | 8 ± 8[c] |
| GDs 18–21 | 48 ± 14 | 50 ± 9 | 38 ± 17 | 26 ± 18[c] |
| GDs 6–21 | 145 ± 27 | 146 ± 19 | 129 ± 32 | 113 ± 30[c] |
| Body weight (g) | | | | |
| GD 21[d] | 337 ± 22 | 327 ± 20 | 314 ± 21 | 297 ± 20[c] |
| Food consumption (g/day) | | | | |
| GDs 6–9 | 20 ± 3 | 19 ± 3 | 17 ± 2[c] | 16 ± 3[c] |
| GDs 18–21 | 22 ± 4 | 23 ± 3 | 21 ± 5 | 18 ± 5[c] |
| AST (U/L) | 61 ± 6 | 76 ± 16 | 76 ± 14 | 91 ± 19[c] |
| ALT (U/L) | 46 ± 8 | 53 ± 6 | 57 ± 13 | 101 ± 18[c] |
| Relative liver weight (percent body weight[d]) | 4.22 ± 0.44 | 4.62 ± 0.38 | 4.95 ± 0.33[c] | 5.40 ± 0.46[c] |
| Hepatic palmitoyl coA oxidase activity (nmol/min/mg proteins) | 14.2 ± 2.8 | 24.8 ± 5.3[c] | 27.0 ± 5.3[c] | 29.6 ± 1.7[c] |

| Table B.5. Significant Changes in Sprague-Dawley Rats Administered DCHP via Gavage on GDs 6–20 | | | | |
|---|---|---|---|---|
| **Parameter** | **Dose (mg/kg-day)** | | | |
| **Litter data** | | | | |
| Fetal body weight (g) | | | | |
| All | 5.63 ± 0.46 | 5.51 ± 0.26 | 5.48 ± 0.29 | 5.00 ± 0.47[c] |
| Males | 5.76 ± 0.44 | 5.65 ± 0.25 | 5.61 ± 0.34 | 5.16 ± 0.50[c] |
| Females | 5.45 ± 0.50 | 5.37 ± 0.27 | 5.32 ± 0.30 | 4.85 ± 0.45[c] |
| AGD; males (mm) | 2.98 ± 0.16 | 2.70 ± 0.16[c] | 2.61 ± 0.15 [c] | 2.47 ± 0.17[c] |
| AGD/body weight$^{1/3}$; males | 1.66 ± 0.07 | 1.52 ± 0.09[c] | 1.47 ± 0.09 [c] | 1.43 ± 0.08[c] |

[a]Mean ± SD.
[b]Significantly different from controls at $p < 0.05$.
[c]Significantly different from controls at $p < 0.01$.
[d]Excluding uterine weight.

Source: Saillenfait et al. (2009).

Yamasaki et al. (2009)

DCHP (99.9% pure) was administered in olive oil at 0, 20, 100, or 500 mg/kg-day to timed-pregnant Crl:CD(SD) IGS rats (10/group) via gavage on GDs 6–20; the dams were allowed to give birth (Yamasaki et al., 2009). These doses were selected on the basis of a preliminary study that showed toxicity and abnormal reproductive performances in dams and offspring at similar dosage levels (data not shown). Mortality and clinical signs of toxicity were monitored daily; body weights were recorded on GDs 0, 6, 13, and 20 and PNDs 4, 7, 14, and 21. The total gestational period for each dam (in days) was calculated. On the day of delivery, the number of live newborns and stillborns and the sex ratio of the pups were recorded, and the pups were examined for external anomalies. The live viability index was calculated on PNDs 4 and 21 (weaning day). Dams were sacrificed the day after weaning. Liver, kidney, thyroid, and ovary weights were recorded. The number of implantation sites in each uterus was counted, and delivery and birth indices were calculated. Pups were monitored daily for clinical signs of toxicity; pup body weights were recorded on PNDs 0, 4, 7, 14, and 21 and weekly thereafter until sacrifice at 10 weeks or later. AGD was measured at PND 4. At PND 13, offspring were examined for retention of thoracic and abdominal nipples. Vaginal opening was monitored in all female rats from PND 21, and preputial separation was examined in all males from PND 35 until it occurred. Prior to weaning, pups were randomly assigned to one of two groups: one group of pups was sacrificed at 10 weeks of age and the other group of pups was used to evaluate reproductive performance of the offspring (Cesarean group).

Endpoints evaluated in pups sacrificed at 10 weeks of age included body weights (recorded weekly and immediately before necropsy), external examinations (number and location of retained nipples, cleft phallus, vaginal pouch, or hypospadias), internal examinations (ectopic or atrophic testes, agenesis of gubernaculums, epididymides and/or sex accessory glands, and epididymal granulomas), estrous cycle (based on vaginal cytology from 8 weeks of age), and organ weights (13 organs). Males and females assigned to the Cesarean group were mated to one another at 12 weeks of age. Copulation and fertility indices were calculated. After Cesarean sections were performed on GD 13, the implantation index and loss were calculated. Necropsies were performed on males on the same day that the Cesarean section was performed on females. The same organs weighed for pups sacrificed at 10 weeks were weighed for the Cesarean group. Regardless of group, all offspring were subjected to histopathological examinations (of the liver, kidneys, testes, epididymides, uterus, ovaries, vagina, pituitary, and thyroids).

Significant treatment-related effects are summarized in Table B.6. One female treated with DCHP at the high dose exhibited dystocia and died on GD 23 (Yamasaki et al., 2009). Body weights of treated rats were not significantly different from controls. Absolute liver weights were reportedly increased in rats treated with DCHP at ≥100 mg/kg-day (data not shown). Statistically significant increases in relative liver weight were also observed in rats treated with 100 or 500 mg/kg-day (increased by 24% at 500 mg/kg-day). The viability index on PND 4 (percent, number of live pups on PND 4/number of live pups on PND 0 [× 100]) was reduced slightly (by 2%) in the 500 mg/kg-day group compared with the control group. The body weights of pups (males and females) were reportedly decreased significantly at 500 mg/kg-day on PNDs 14 and/or 21 (data not shown). Also with regard to pups, two males from the high-dose group showed hypospadia accompanied by small testes; one of these animals was sacrificed at 7 weeks of age because of poor general condition. With respect to controls, preputial separation was prolonged by 5%, AGD on PND 4 was decreased by 15%, and nipple retention/of areola was increased by 68% in males treated at 500 mg/kg-day (data for other dose groups not shown); no significant effects with respect to these parameters were reported for the lower dose groups. No abnormalities in vaginal opening day or estrous cycle were observed in treated females. Other than decreased relative muscle weight and slight histological changes (including decreased testicular germ cells and degenerated proximal tubules; incidence data not shown) in male rats treated at 500 mg/kg-day, treated pups sacrificed at 10 weeks of age were comparable to controls. No changes in the reproductive parameters were detected in any DCHP-exposed pups from the Cesarean group. Significant changes in thymus, spleen, and brain weights in F1 and F2 offspring were attributed by the authors to inhibition of body weight gain, since changes

occurred only for either absolute or relative weights, and some showed contrary results between the two.

**Table B.6. Significant Effects in Crl:CD(SD) IGS Rats Treated with DCHP via Gavage on GDs 6–20**

| Parameter | Dose (mg/kg-d) | | | |
|---|---|---|---|---|
| | 0 | 20 | 100 | 500 |
| Body weight (g) | 482.1 ± 37.2[a] | 475.7 ± 56.7 | 496.2 ± 33.2 | 468.1 ± 37.6 |
| Relative liver weight (percent body weight) | 4.65 ± 0.29 | 4.82 ± 0.32 | 4.97 ± 0.25[b] | 5.75 ± 0.17[b] |
| Viability index on PND 4 (percent) | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 97.8 ± 3.3[b] |
| Age preputial separation (days) | 43.5 ± 2.2 | Not reported | Not reported | 45.6 ± 2.3[b] |
| AGD (mm) | 4.23 ± 0.39 | Not reported | Not reported | 3.59 ± 0.32[b] |
| Incidence areolas/nipples retention (percent) | 0.0 ± 0.0 | Not reported | Not reported | 67.6 ± 40.5[b] |
| Offspring: relative muscle weight (percent body weight) | 0.22 ± 0.02 | 0.20 ± 0.04 | 0.21 ± 0.03 | 0.20 ± 0.02[b] |

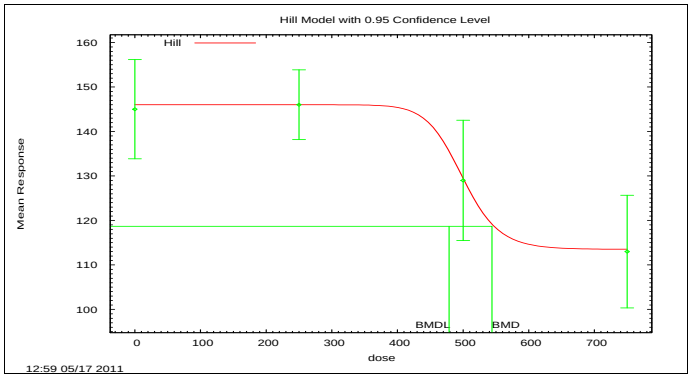
[a]Mean ± SD.
[b]Significantly different from controls at $p < 0.05$.

Source: Yamasaki et al. (2009).

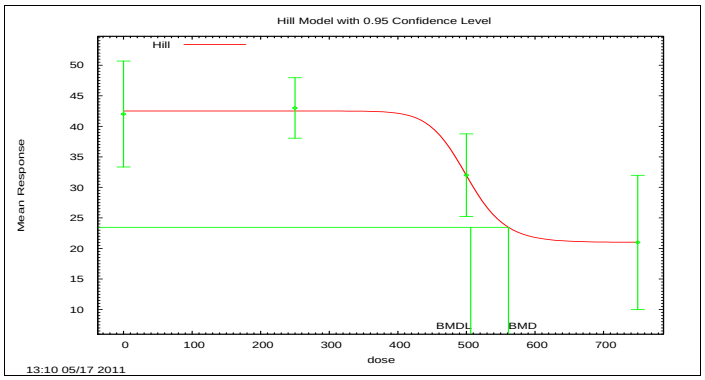
Based on these data, parental NOAEL and LOAEL values of 20 and 100 mg/kg-day for increased relative liver weight are identified. A developmental LOAEL of 500 mg/kg-day is identified based on slightly reduced viability index (PND 4); decreased pup body weight of male and female pups on PND 14/21; and prolonged preputial separation, decreased AGD, increased incidence of areola/nipple retention, and slight histological changes (testis and kidney) in male pups; 100 mg/kg-day is identified as a NOAEL since no effects on these endpoints were reported for this dose group.

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

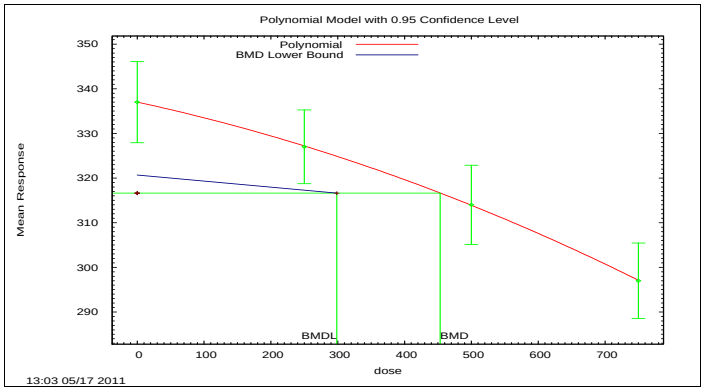
| Maternal Body Weight (Gavage; Gd 6-21; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 544 | 479 | 0.9 | 751 |
| Linear | 604 | 438 | 0.21 | 752 |
| Polynomial | 673 | 513 | 0.43 | 751 |
| Power | 664 | 506 | 0.42 | 751 |



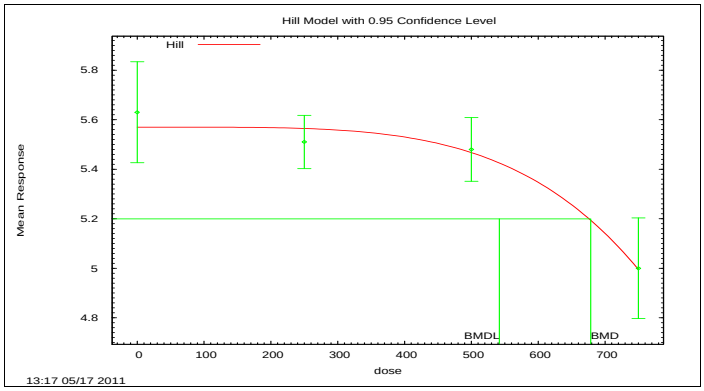

| Maternal Body Weight Corrected for Gravid Uterus Weight (Gavage; Gd 6-21; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 561 | 506 | 0.85 | 683 |
| Linear | 655 | 465 | 0.23 | 684 |
| Polynomial | 701 | 543 | 0.49 | 684 |
| Power | 692 | 533 | 0.46 | 684 |



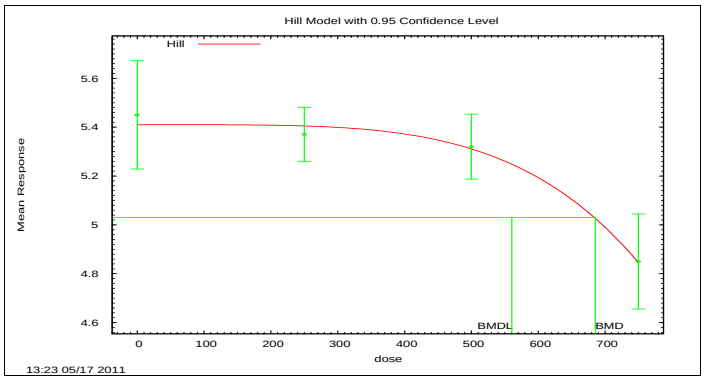
| Maternal Body Weight Corrected for Gravid Uterus Weight (Gavage; Gd 21; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 450 | 312 | na | 699 |
| Linear | 384 | 306 | 0.7 | 695 |
| Polynomial | 453 | 299 | 0.96 | 697 |
| Power | 450 | 314 | 0.87 | 697 |



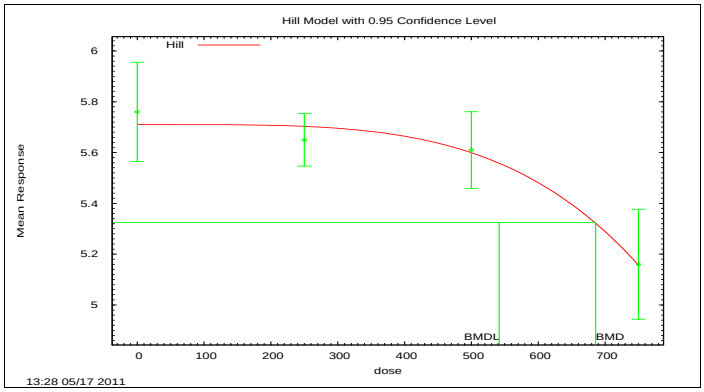

| All Fetal Body Weight (Gavage; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 678 | 542 | na | -79 |
| Linear | 499 | 374 | 0.03 | -77 |
| Polynomial | 630 | 492 | 0.2 | -80 |
| Power | 678 | 542 | 0.29 | -81 |



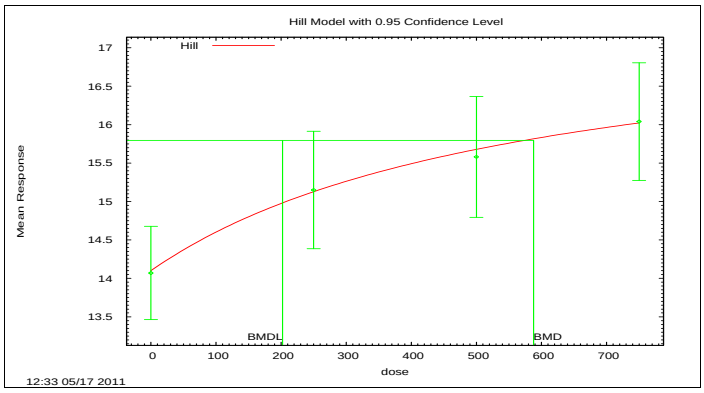
| Female Fetal Body Weight (Gavage; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 685 | 561 | na | -75 |
| Linear | 529 | 392 | 0.02 | -72 |
| Polynomial | 653 | 522 | 0.2 | -76 |
| Power | 685 | 561 | 0.5 | -77 |



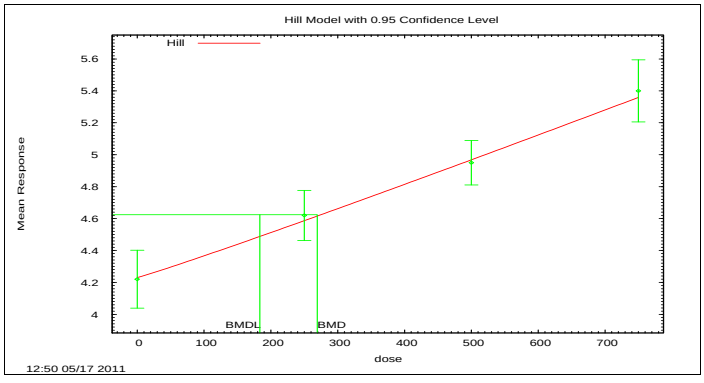

| Male Fetal Body Weight (Gavage; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 686 | 542 | na | -73 |
| Linear | 553 | 394 | 0.05 | -73 |
| Polynomial | 647 | 503 | 0.18 | -74 |
| Power | 686 | 542 | 0.35 | -76 |



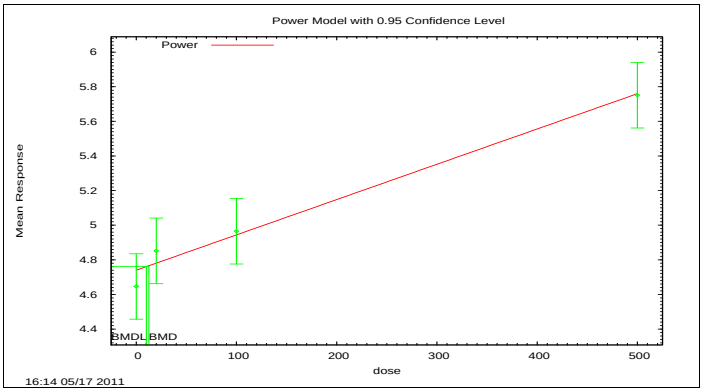
| Maternal Absolute Liver Weight (Gavage; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 588 | 202 | 0.78 | 212 |
| Linear | 679 | 478 | 0.61 | 211 |
| Polynomial | 581 | 281 | 0.66 | 212 |
| Power | 679 | 478 | 0.61 | 211 |



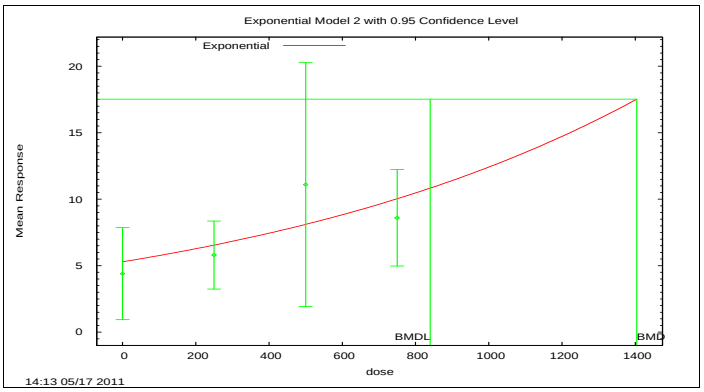

| Maternal Relative Liver Weight (Gavage; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 269 | 183 | na | -73 |
| Linear | 257 | 216 | 0.83 | -77 |
| Polynomial | 273 | 190 | 0.59 | -75 |
| Power | 269 | 216 | 0.57 | -75 |



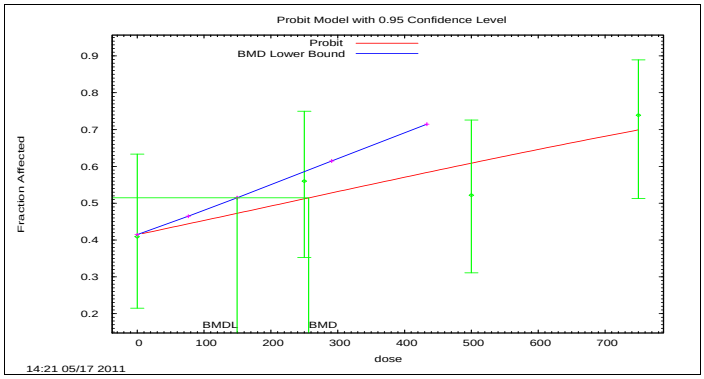
| Maternal Relative Liver Weight (Gavage; Yamasaki et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 8.4 | 3.9 | 0.19 | -61 |
| Linear | 13 | 10 | 0.33 | -62 |
| Polynomial | 8.9 | 5 | 0.18 | -61 |
| Power | 13 | 10 | 0.33 | -62 |



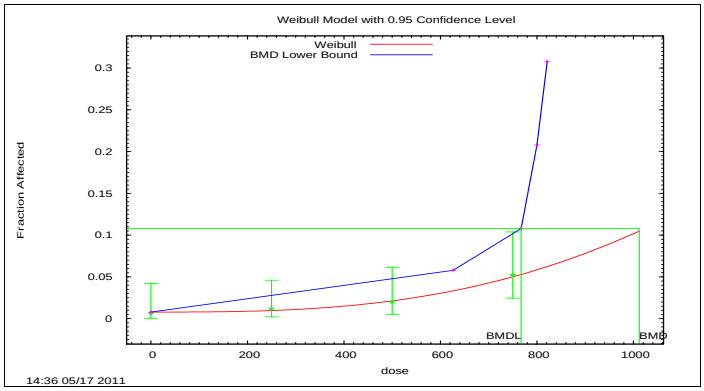

| Maternal Post-implantation Loss/Litter (Gavage; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | . | . | 0.48 | 565 |
| Linear | 1705 | 827 | 0.44 | 565 |
| Polynomial | -9999 | 876 | 0.29 | 566 |
| Power | 1705 | 827 | 0.44 | 565 |
| Exponential $2^{o}$ | 1404 | 840 | 0.38 | 565 |



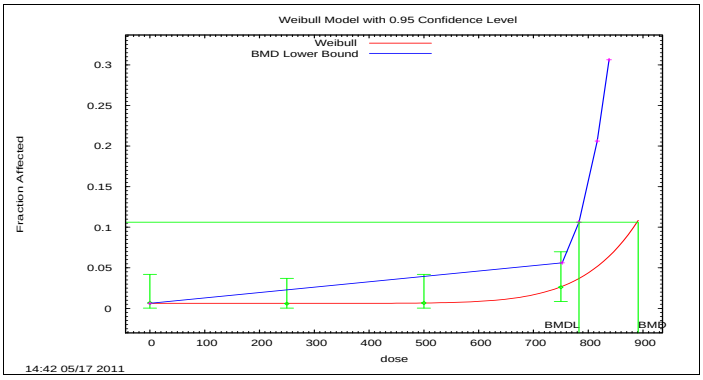
| Litters with Resorptions (Gavage; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Gamma | 602 | 101 | 0.3 | 129 |
| Logistic | 256 | 149 | 0.6 | 127 |
| Multistage | 327 | 101 | 0.3 | 129 |
| Probit | 256 | 149 | 0.57 | 127 |
| Weibull | 598 | 101 | 0.3 | 129 |



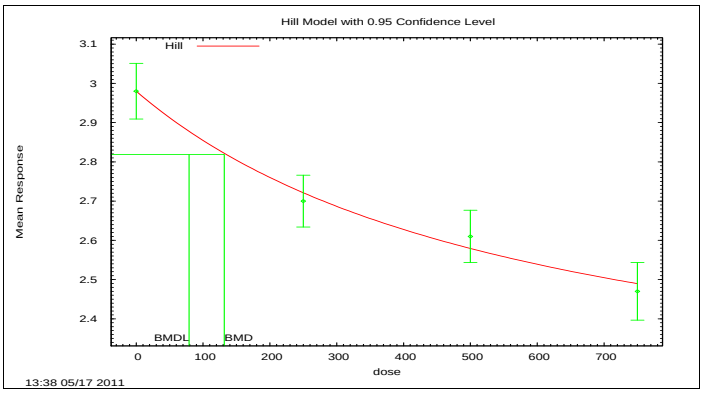

| Incidence of Cervical Ribs (per fetus) (Gavage; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Gamma | 1038 | 788 | 0.71 | 132 |
| Logistic | 1020 | 809 | 0.9 | 130 |
| Multistage | 1176 | 893 | 0.89 | 130 |
| Probit | 1086 | 831 | 0.87 | 130 |
| Weibull | 1012 | 767 | 0.72 | 132 |



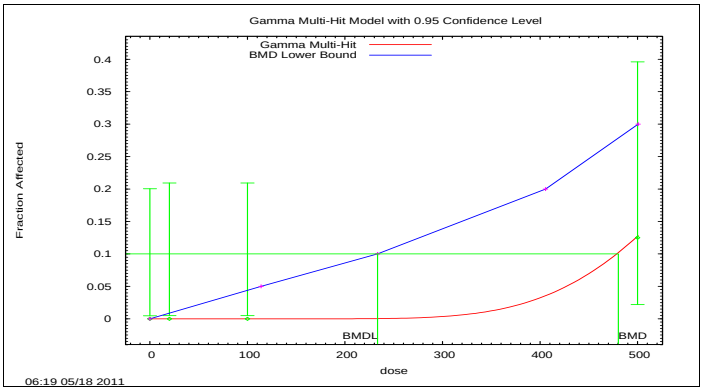
| Incidence of Cervical Ribs (per litter) (Gavage; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Gamma | 936 | 832 | 0.93 | 79 |
| Logistic | 1453 | 947 | 0.7 | 78 |
| Multistage | 1877 | 1234 | 0.71 | 78 |
| Probit | 1660 | 1021 | 0.63 | 78 |
| Weibull | 891 | 783 | 0.93 | 79 |



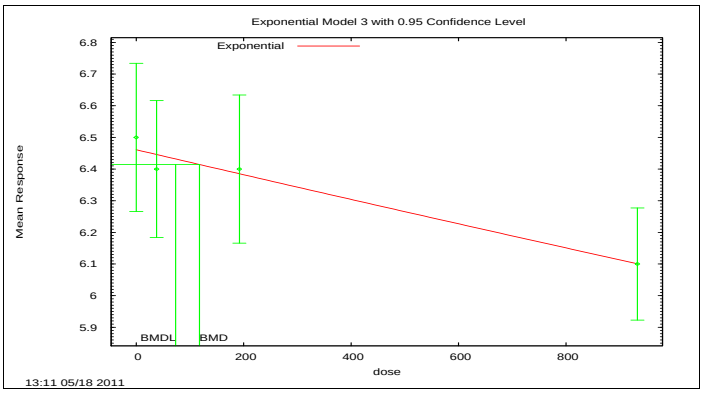

| Male Fetus Anogenital Distance (mm) (Gavage; Saillenfait et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 132 | 79 | 0.2 | 239 |
| Linear | 254 | 211 | 0.03 | 236 |
| Polynomial | 161 | 120 | 0.1 | 238 |
| Power | 254 | 211 | 0.026 | 236 |



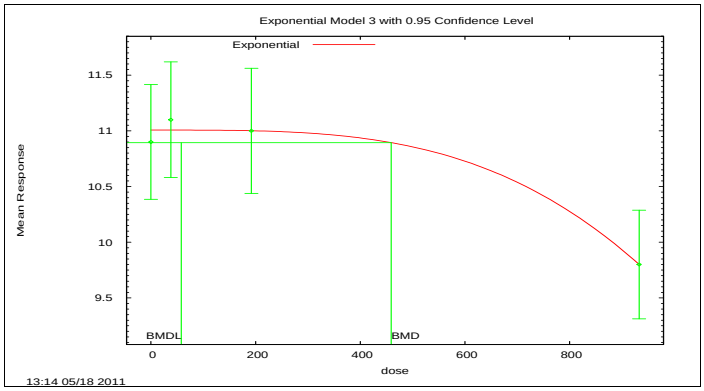
| Incidence of Male Pup Hypospadias (Gavage; Yamasaki et al., 2009) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Gamma | 480 | 233 | 1 | 16 |
| Logistic | 494 | 367 | 1 | 16 |
| Multistage | 515 | 194 | 0.89 | 15 |
| Probit | 489 | 343 | 1 | 16 |
| Weibull | 489 | 234 | 1 | 16 |



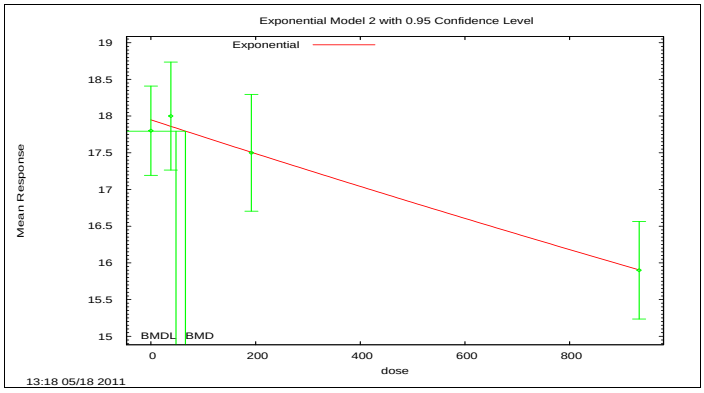

| F1 Female Pup Body Weight (Dietary; D0; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 120 | 76 | 0.54 | -37 |
| Linear | 121 | 77 | 0.83 | -39 |
| Polynomial | 124 | 26 | 0.54 | -37 |
| Power | 121 | 77 | 0.83 | -39 |
| Exponential $3^{o}$ | 118 | 73 | 0.8 | -39 |



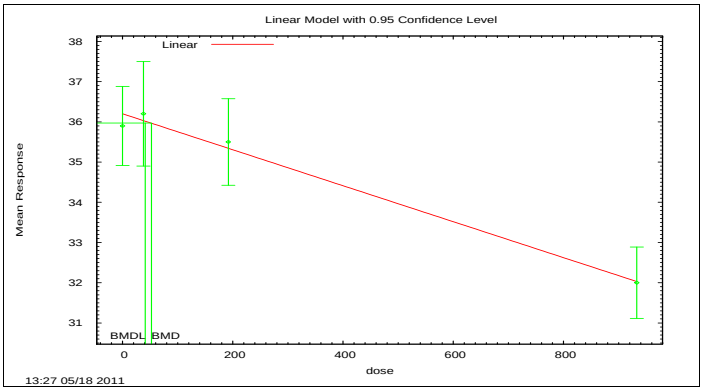
| F1 Female Pup Body Weight (Dietary; D4; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 296 | 34 | na | 115 |
| Linear | 84 | 59 | 0.58 | 112 |
| Polynomial | 431 | 38 | 0.59 | 113 |
| Power | 464 | 62 | 0.56 | 113 |
| Exponential $3^o$ | 459 | 58 | 0.6 | 113 |



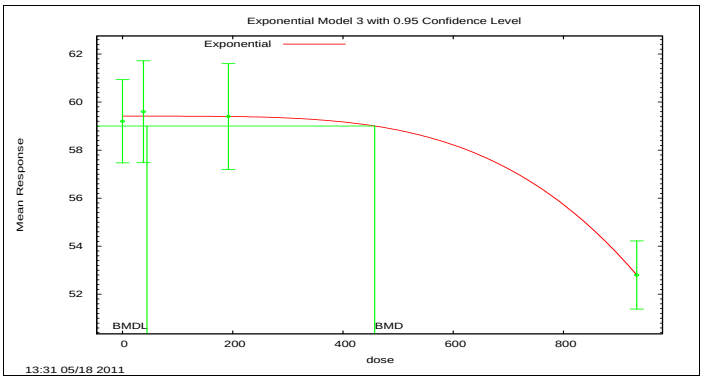

| F1 Female Pup Body Weight (Dietary; D7; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 173 | 18 | na | 167 |
| Linear | 70 | 52 | 0.83 | 163 |
| Polynomial | 76 | 23 | 0.54 | 165 |
| Power | 88 | 52 | 0.55 | 165 |
| Exponential $2^o$ | 66 | 48 | 0.83 | 163 |



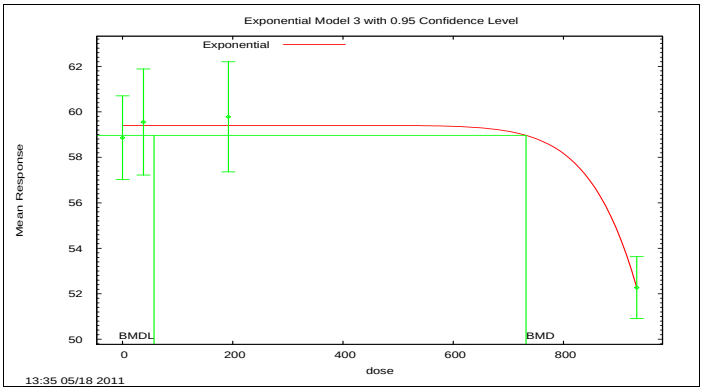
| F1 Female Pup Body Weight (Dietary; D14; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 175 | 22 | na | 240 |
| Linear | 53 | 41 | 0.76 | 236 |
| Polynomial | 93 | 24 | 0.57 | 238 |
| Power | 105 | 42 | 0.6 | 238 |



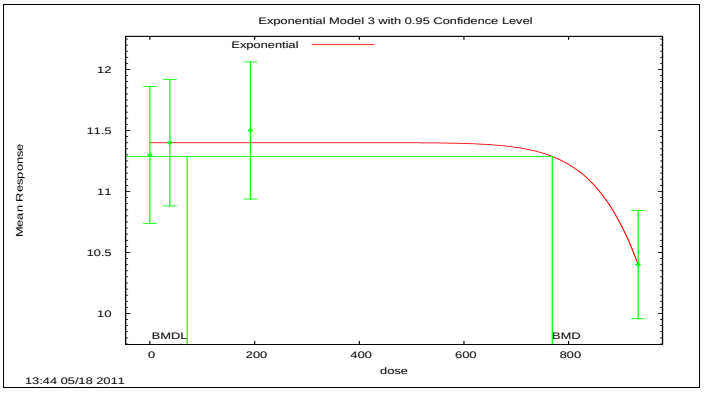

| F1 Female Pup Body Weight (Dietary; D21; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 364 | 38 | na | 335 |
| Linear | 56 | 43 | 0.44 | 332 |
| Polynomial | 355 | 39 | 0.79 | 333 |
| Power | 461 | 47 | 0.75 | 333 |
| Exponential $3^{o}$ | 457 | 44 | 0.8 | 333 |



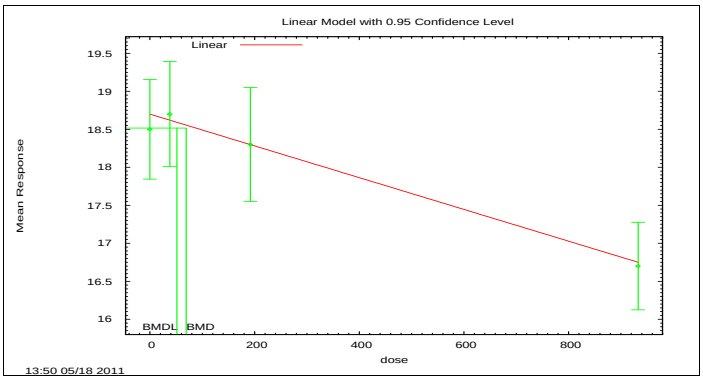
| F1 Female Pup Body Weight (Dietary; Final; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 701 | 56 | na | 347 |
| Linear | 57 | 44 | 0.21 | 346 |
| Polynomial | 512 | 61 | 0.73 | 345 |
| Power | 743 | 55 | 0.49 | 345 |
| Exponential $3^o$ | 732 | 57 | 0.5 | 345 |



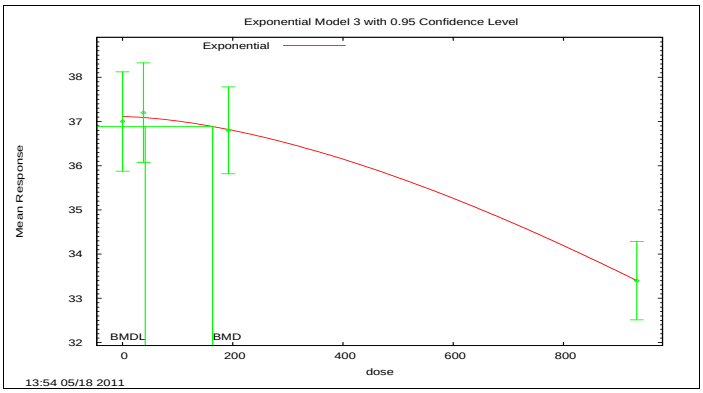

| F1 Male Pup Body Weight (Dietary; D4; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 751 | 46 | na | 115 |
| Linear | 103 | 69 | 0.49 | 112 |
| Polynomial | 625 | 54 | 0.89 | 113 |
| Power | 732 | 75 | 0.57 | 113 |
| Exponential $3^o$ | 767 | 71 | 0.6 | 113 |



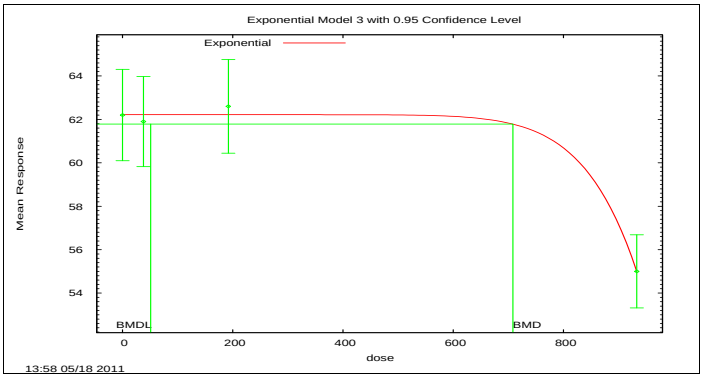
| F1 Male Pup Body Weight (Dietary; D7; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 177 | 21 | na | 158 |
| Linear | 69 | 51 | 0.81 | 154 |
| Polynomial | 104 | 25 | 0.56 | 156 |
| Power | 116 | 52 | 0.58 | 156 |



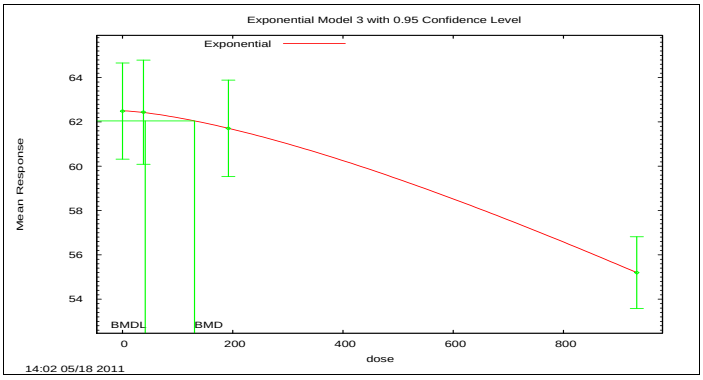

| F1 Male Pup Body Weight (Dietary; D14; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 186 | 27 | na | 232 |
| Linear | 55 | 43 | 0.7 | 229 |
| Polynomial | 166 | 29 | 0.72 | 230 |
| Power | 163 | 44 | 0.74 | 230 |
| Exponential $3^{o}$ | 163 | 41 | 0.8 | 230 |



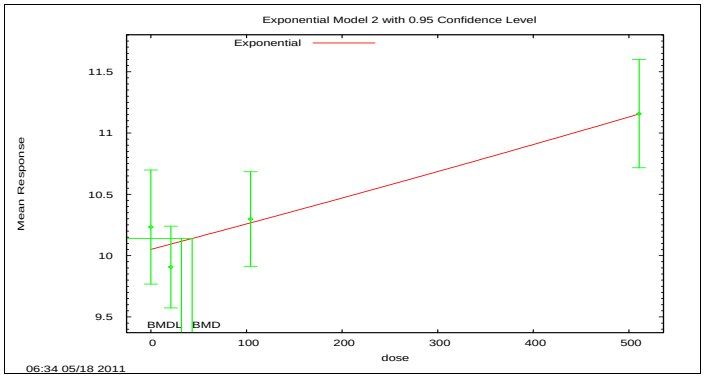
| F1 Male Pup Body Weight (Dietary; D21; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 692 | 50 | na | 345 |
| Linear | 55 | 43 | 0.25 | 343 |
| Polynomial | 466 | 52 | 0.7 | 342 |
| Power | 725 | 51 | 0.6 | 343 |
| Exponential $3^{o}$ | 708 | 51 | 0.6 | 343 |



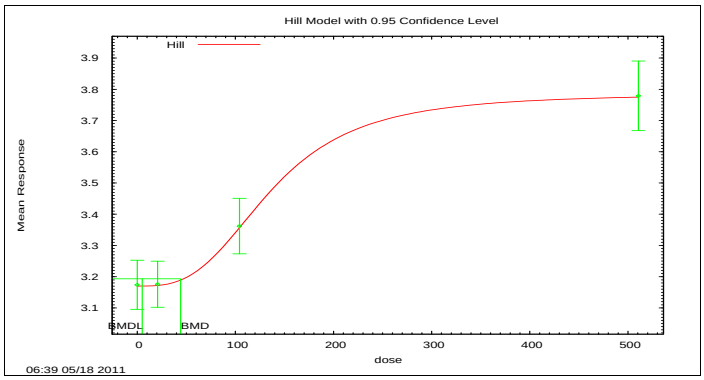

| F1 Male Pup Body Weight (Dietary; Final; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 139 | 23 | na | 352 |
| Linear | 56 | 44 | 0.86 | 348 |
| Polynomial | 118 | 26 | 0.95 | 350 |
| Power | 129 | 44 | 0.98 | 350 |
| Exponential $3^{o}$ | 130 | 41 | 1 | 350 |



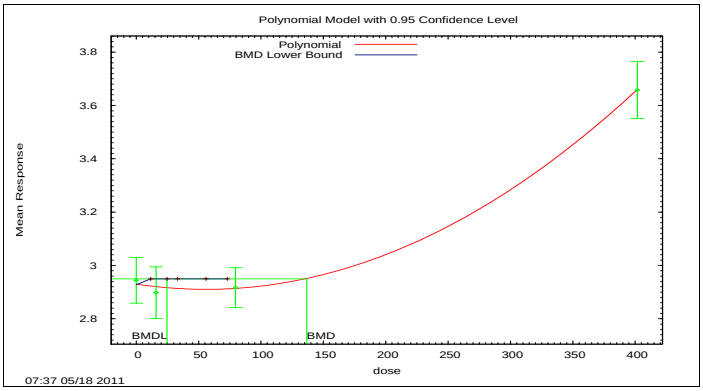
| F0 Female Absolute Liver Weight (Dietary; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 94 | 12 | na | 74 |
| Linear | 41 | 30 | 0.38 | 71 |
| Polynomial | 53 | 13 | 0.17 | 73 |
| Power | 63 | 30 | 0.18 | 73 |
| Exponential $2^{o}$ | 43 | 32 | 0.39 | 71 |



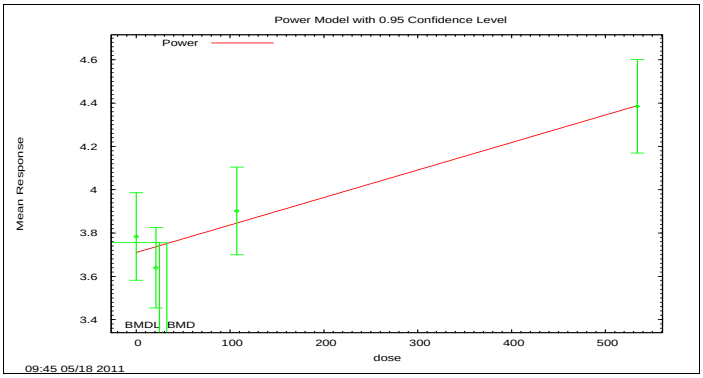

| F0 Female Relative Liver Weight (Dietary; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 44 | 5.1 | na | 184 |
| Linear | 17 | 14 | 0.32 | 186 |
| Polynomial | 9.3 | 6 | 0.47 | 186 |
| Power | 17 | 14 | 0.31 | 186 |



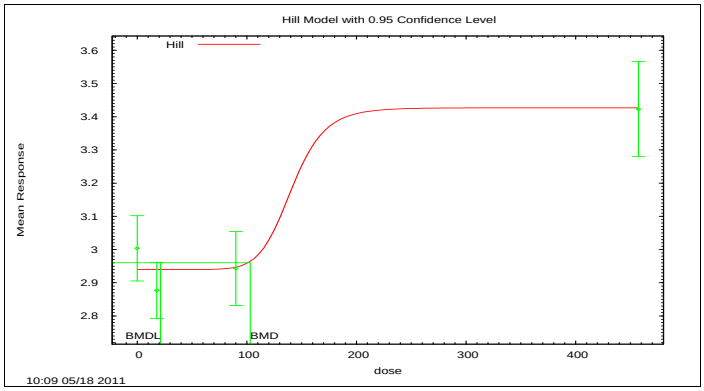
| F0 Male Relative Liver Weight (Dietary; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 271 | 29 | na | -190 |
| Linear | 12 | 9.9 | 0.01 | -187 |
| Polynomial | 137 | 25 | 0.54 | -193 |
| Power | 287 | 29 | 0.45 | -193 |



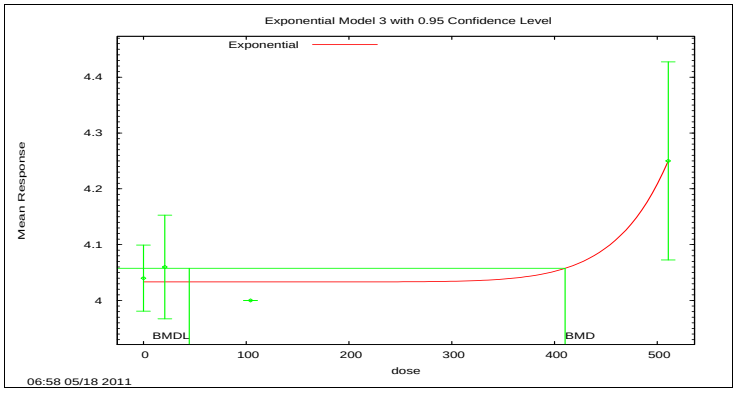

| F1 Female Relative Liver Weight (Dietary; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 91 | 9.3 | na | -50 |
| Linear | 33 | 25 | 0.34 | -53 |
| Polynomial | 24 | 10 | 0.15 | -51 |
| Power | 33 | 25 | 0.34 | -53 |



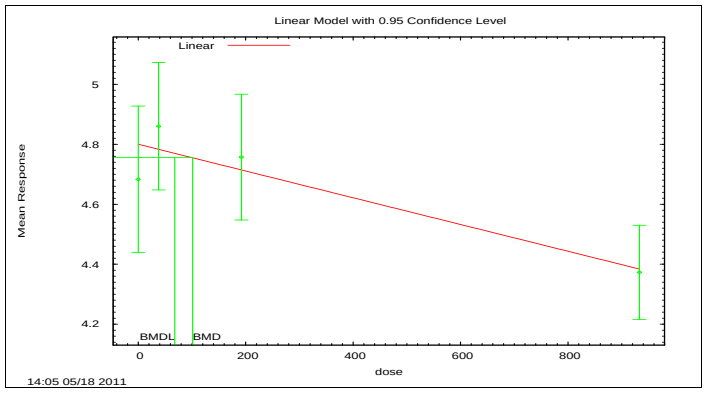
| F1 Male Relative Liver Weight (Dietary; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 103 | 21 | na | -144 |
| Linear | 22 | 18 | 0.06 | -146 |
| Polynomial | 197 | 23 | 0.1 | -147 |
| Power | 158 | 21 | 0.08 | -147 |



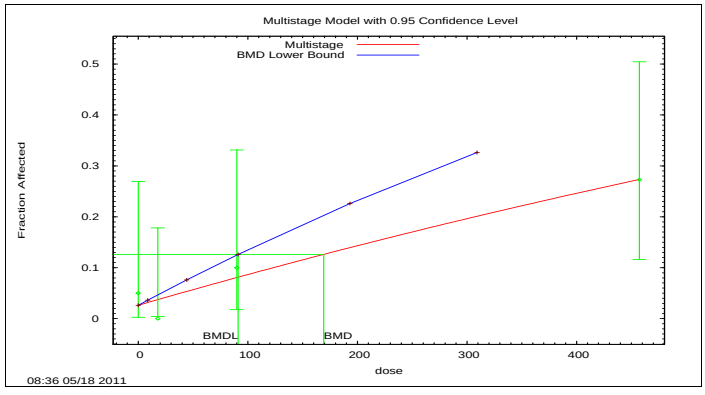

| F0 Female Mean Estrous Cycle (Dietary; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 416 | 33 | na | -166 |
| Linear | 57 | 39 | 0.32 | -168 |
| Polynomial | 362 | 41 | 0.62 | -168 |
| Power | 408 | 43 | 0.38 | -168 |
| Exponential $3^{o}$ | 410 | 45 | 0.4 | -168 |



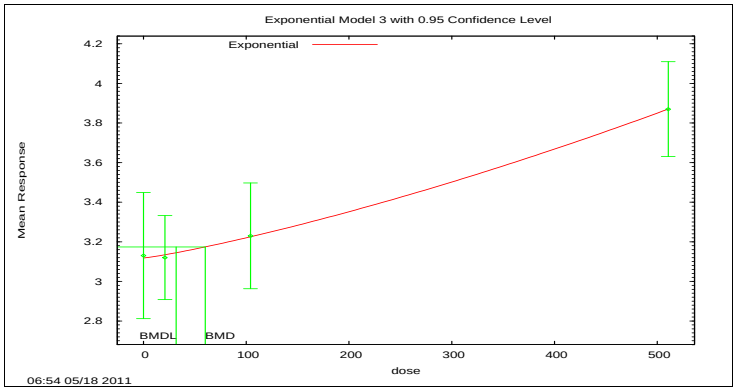
| F1 Male Pup Anogenital Distance (mm) (Dietary; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 209 | 33 | na | -41 |
| Linear | 101 | 68 | 0.33 | -44 |
| Polynomial | 472 | 38 | 0.2 | -43 |
| Power | 344 | 70 | 0.2 | -42 |



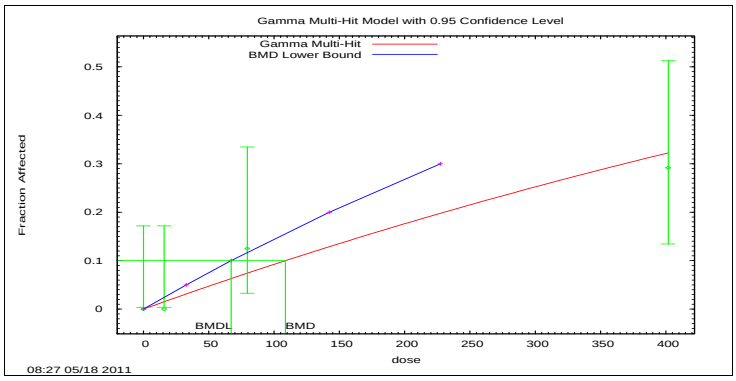

| F1 Male Incidence of Testicular Atrophy and Seminiferous Tubule Pathology (Dietary; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Gamma | 186 | 92 | 0.23 | 55 |
| Logistic | 281 | 205 | 0.4 | 53 |
| Multistage | 169 | 91 | 0.49 | 53 |
| Probit | 264 | 188 | 0.44 | 53 |
| Weibull | 186 | 92 | 0.23 | 55 |



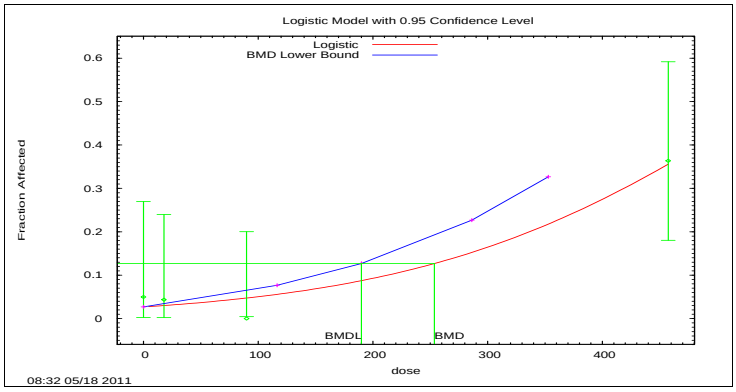
| F0 Female Relative Left Thyroid Weight (Dietary; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 97 | 11 | na | -4.5 |
| Linear | 37 | 28 | 0.95 | -8.4 |
| Polynomial | 56 | 13 | 0.86 | -6.5 |
| Power | 61 | 28 | 0.88 | -6.5 |
| Exponential $3^{o}$ | 60 | 32 | 0.9 | -6.4 |




| F0 Male Incidence of Hypertrophic Follicular Cells in Thyroid (Dietary; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Gamma | 109 | 67 | 0.71 | 51 |
| Logistic | 239 | 180 | 0.1 | 56 |
| Multistage | 109 | 67 | 0.71 | 51 |
| Probit | 221 | 164 | 0.11 | 56 |
| Weibull | 109 | 67 | 0.71 | 51 |



| F1 Male Incidence of Kidney Pathology (Dietary; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Gamma | 365 | 131 | 0.32 | 53 |
| Logistic | 254 | 190 | 0.5 | 51 |
| Multistage | 146 | 82 | 0.27 | 53 |
| Probit | 233 | 172 | 0.44 | 52 |
| Weibull | 403 | 131 | 0.32 | 53 |




| F1 Female Pup Brain Weight (Dietary; Hoshino et al., 2005) | | | | |
|---|---|---|---|---|
| **Model** | **$BMD_{10}$** | **$BMDL_{10}$** | **P-value** | **AIC** |
| Hill | 724 | . | na | -454 |
| Linear | 126 | 79 | 0.32 | -458 |
| Polynomial | 220 | 29 | 0.14 | -456 |
| Power | 705 | 79 | 0.13 | -456 |
| Exponential $3^{o}$ | 125 | 77 | 0.3 | -458 |