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Provisional Peer Reviewed Toxicity Values for

2,4-Dichlorophenol
(CASRN 120-83-2)

Superfund Health Risk Technical Support Center
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Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor

p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR 2,4-DICHLOROPHENOL (CASRN 120-83-2)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and

circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

A chronic reference dose (RfD) for 2,4-dichlorophenol (2,4-DCP) is available on IRIS (U.S. EPA, 2007). The RfD of 0.003 mg/kg-day is based on decreased delayed hypersensitivity response in a rat study (Exon and Koller, 1985). Rats were exposed during gestation, through lactation and in drinking water for 15 weeks and a NOEL of 0.3 mg/kg-day was identified (Exon and Koller, 1985). Uncertainty factors of 10 each for interspecies extrapolation and protection of sensitive humans were applied to the NOEL to derive the RfD. The source document was a U.S. EPA (1985) Drinking Water Criteria Document (DWCD). The Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) includes the same chronic RfD of 0.003 mg/kg-day as reported on IRIS. The HEAST (U.S. EPA, 1997) reports a subchronic RfD of 0.003 mg/kg-day for 2,4-DCP, adopting the chronic RfD from IRIS as the subchronic RfD. ATSDR (1999) has prepared a toxicological profile for chlorophenols. An intermediate-duration oral Minimal Risk Level (MRL) of 0.003 mg/kg-day was derived for 2,4-DCP by ATSDR (1999). The MRL is based on the same study, endpoint, and uncertainty factors as the IRIS chronic RfD. The World Health Organization (WHO, 1989, 2003) evaluated the toxicity of 2,4-DCP. WHO (2003) declined to derive health-based guideline values, citing limitations in the toxicity database. Because a chronic RfD is available on IRIS, the present document does not include a chronic provisional-RfD (p-RfD); however, a subchronic p-RfD is included.

An RfC for 2,4-DCP is not available on IRIS (U.S. EPA, 2007) or in the HEAST (U.S. EPA, 1997). ATSDR (1999) did not derive any inhalation MRLs for 2,4-DCP, as there were no inhalation toxicity studies of this compound. The CARA list (U.S. EPA, 1991, 1994) includes a Health and Environmental Assessment (HEA) for 2,4-DCP (U.S. EPA, 1987a) and a Health and

Environmental Effects Document (HEED) for Chlorinated Phenols (U.S. EPA, 1987b), in addition to the DWCD (U.S. EPA, 1985) cited above, but none of these documents reported pertinent data regarding subchronic or chronic inhalation toxicity. The American Conference of Governmental Industrial Hygienist (ACGIH, 2006), Occupational Safety and Health Administration (OSHA, 2006) and National Institute for Occupational Safety and Health (NIOSH, 2006) have not established occupational health standards for 2,4-DCP.

A carcinogenicity assessment for 2,4-DCP is not available on IRIS (U.S. EPA, 2007) or in the HEAST (U.S. EPA, 1997). The Drinking Water Standards and Health Advisories document classifies the carcinogenicity of 2,4-DCP in category E, evidence of noncarcinogenicity for humans (U.S. EPA, 2006). The International Agency for Research on Cancer (IARC, 1999) concluded that there was *evidence suggesting lack of carcinogenicity* for 2,4-DCP in experimental animals based on an oral study in mice and two oral studies in rats (NTP, 1989; Exon and Koller, 1985). 2,4-DCP is not included in the National Toxicology Program's (NTP) 11th Report on Carcinogens (NTP, 2006). Patty's Toxicology (Gingell et al., 2001) was also consulted for relevant information.

To identify toxicological information pertinent to the derivation of provisional toxicity values for 2,4-DCP, searches were conducted in August, 2006 for literature dating from the 1960s to 2006 using the following databases: MEDLINE , TOXLINE, BIOSIS, TSCATS CCRIS, Current Contents, DART/ETIC, GENETOX, HSDB and RTECS.

REVIEW OF PERTINENT DATA

Human Studies

Studies exist regarding the toxicity of chlorophenol mixtures in humans, but neither the literature search nor available reviews (U.S. EPA, 1987a,b; ATSDR, 1999; IARC, 1986, 1999; WHO, 1989) identified any studies regarding the toxicity of 2,4-DCP as a single agent in humans. Among workers in a 2,4-DCP and 2,4,5-trichlorophenol manufacturing plant, chloracne and porphyria were detected (Bleiberg et al., 1964). Elevated serum transaminase levels and evidence of liver damage (regeneration and hemofuscin deposition) were detected by liver biopsy in two cases. Poland et al. (1971) examined employees from the same plant 6 years after the report of Bleiberg et al. (1964). Of the 73 male workers examined, 48 (66%) had some degree of acne, and chloracne was found in 13 workers (18%); no cases of clinical porphyria were documented and only one worker had uroporphyrinuria. The severity of the chloracne was not correlated with job location within the plant or duration of employment. A causal relationship between these effects and exposure to 2,4-DCP in these workers cannot be assumed due to concurrent exposure to a variety of chlorinated compounds.

The literature search did not identify any studies regarding carcinogenicity of 2,4-DCP as a single agent in humans. There are several case-control (Eriksson et al., 1981; Hardell, 1981; Hardell et al., 1981, 1982; Hardell and Sandstrom, 1979) and cohort studies (Axelson et al., 1980, Bueno de Mesquita et al., 1993; Hogsted and Westerlund, 1980; Kogevinas et al., 1992, 1993; Saracci et al., 1991; Lynge, 1987; Riihimaki et al., 1982, 1983; U.S. Air Force, 1983;

Vena et al., 1998) of workers involved in the manufacture of phenoxy herbicides based on 2,4-DCP. In these studies, workers were exposed to a mixture of chemicals including 2,4-DCP and it was not possible to link mortality or tumor incidence to any particular chemical exposure in any of these studies. A follow up of a cohort study to investigate the potential carcinogenic effect of phenoxy herbicides (e.g., 2,4-dichlorophenol) in Danish workers (Lynge, 1985) reported soft tissue sarcomas in male workers. However, the total cancer risk among persons employed in the manufacturing and packaging of phenoxy herbicides was equivalent to the cancer risk in the Danish population. Furthermore, this study had several potential biases, such as exposure to mixtures of other chemicals. Therefore, observed cancer incidences cannot be linked to 2,4-dichlorophenol.

Morbidity and Mortality Weekly Report (MMWR, 2000) reported 5 human fatalities associated with acute exposure to 2,4-DCP in occupational settings. In each of these cases, dermal exposure was significant and chemical burns were common. Inhalation exposure was possible in several of the cases, but the proportion of dose attributable to inhalation exposure could not be determined (MMWR, 2000). Kintz et al. (1992) also reported a fatality associated with dermal exposure to 2,4-DCP. After spilling the pure compound on his right thigh and arm, a 33-year old man experienced seizures and died. His blood level of 2,4-DCP was measured to be 24.3 mg/L.

Animal Studies

Oral Exposure

Subchronic Exposure — In a subchronic study using CD-1 mice, Borzelleca et al. (1985a) administered 2,4-DCP (99% pure) in drinking water to groups of 20 male and 20 female mice for 90 days. Concentrations of 0.2, 0.6 or 2.0 mg/L in 10% Emulphor were added to the drinking water of treated animals; two control groups received either vehicle or deionized water. Water was provided *ad libitum* and intake was measured twice weekly. Clinical observations were made twice daily and body weights were measured weekly. At study termination, surviving mice were sacrificed and necropsied. Blood was collected for hematology (erythrocyte count, leukocyte count [total and differential], platelet count, hematocrit, hemoglobin [Hb], prothrombin and thromboplastin times, plasma fibrinogen) and clinical chemistry (aspartate aminotransferase [AST], alanine aminotransferase [ALT], lactate dehydrogenase [LDH], alkaline phosphatase [ALP], blood urea nitrogen [BUN], glucose, bilirubin, albumin, total protein, cholesterol, creatinine, phosphorus, calcium, globulin, albumin/globulin ratio and electrolytes). Hepatic microsomal enzyme activities (ethoxycoumarin O-deethylase, testosterone hydroxylation, cytochrome p-450 reductase) were also measured. Brain, liver, spleen, lung, thymus and kidney weights were recorded. No tissues were examined histologically.

Based on measured water consumption and body weights, the authors estimated doses of 0, 40, 114 and 383 mg/kg-day for male mice and 0, 50, 143 and 491 mg/kg-day for female mice (Borzelleca et al., 1985a). Treatment with 2,4-DCP did not result in significant differences in body weight, absolute or relative organ weights or microsomal enzyme activity when compared with the vehicle control; however, the vehicle itself (Emulphor) apparently increased body weight and altered some organ weights compared with the naïve controls. Among male rats

treated at the high dose, there was a significant increase in leukocytes (35% higher than vehicle control, $p \leq 0.05$). In females at the high dose, a significant increase in ALP was observed (1.7-fold higher, $p \leq 0.05$). The significance of these changes is uncertain in the absence of toxicological correlates. No other changes in hematology or clinical chemistry were dose-related. Effect levels cannot be identified from these data due to the confounding effect of the vehicle and the absence of histopathological evaluation.

The NTP sponsored a 13-week study to evaluate the toxic effects of subchronic oral exposure to 2,4-DCP and to determine the appropriate doses to be used in a 2-year study (NTP, 1989). F344/N rats and B6C3F1 mice (10/sex) were given 2,4-DCP (>99% pure) in the diet at concentrations of 0, 2500, 5000, 10,000, 20,000 or 40,000 ppm. Clinical observations were performed twice daily; body weight and food consumption were measured twice during the study. After 13 weeks, all animals were subjected to necropsy and histological examination of a comprehensive list of tissues (>40) was conducted on the control and high-dose animals. In the 10,000 ppm and 20,000 ppm rats, the bone marrow, colon, heart, jejunum, stomach and urinary bladder were evaluated histologically; the femoral bone marrow was also examined in 2500 ppm and 5000 ppm female rats. Histologic examination of the liver was performed in 2500, 5000 and 10,000 ppm mice of both sexes.

Using limited data on food consumption and body weight from the report (NTP, 1989), doses in rats can be estimated as 160, 310, 675, 1373 and 2703 mg/kg-day in males and 182, 338, 750, 1376 and 2795 mg/kg-day in females. All rats survived to study termination. Body weights decreased in a dose-related fashion in both sexes. Terminal body weight was significantly lower than controls in males exposed to 10,000 ppm and higher ($p<0.01$ in t-test performed for this review). Terminal body weight was lower than controls by 5%, 20% and 40% at 10,000, 20,000, and 40,000 ppm, respectively. In females, terminal body weight was significantly below control values at concentrations of 20,000 and 40,000 ppm (11% and 21% lower). Average food consumption (measured on weeks 7 and 13) was decreased to 77% and 81% of control values in males and females exposed to 40,000 ppm. Statistical comparison of the food consumption rates was not reported. Rats exposed to 40,000 ppm exhibited hunched posture and rough hair coats. At necropsy, histopathology evaluations revealed bone marrow atrophy in all animals of both sexes exposed to the two highest concentrations and in females (6/10) of the 10,000 ppm group. The incidence of bone marrow atrophy in controls and lower dose groups, if any, was not reported. No other histopathology findings were reported. This study identifies a LOAEL of 10,000 ppm (750 mg/kg-day) for bone marrow atrophy in females. Male rats treated at this concentration also had slight reductions in body weight (5%). The 5000 ppm concentration (338 mg/kg-day) represents a NOAEL.

Based on limited data on food consumption and body weight from the report (NTP, 1989), doses in mice[1] can be estimated as 782, 1533, 1627, 2960 and 6805 mg/kg-day in males and 973, 2438, 3305, 3913 and 8911 mg/kg-day in females. Among high-dose mice, mortality was 100% within the first 3 weeks on study; survival was not different from controls in other

[1] Two measurements of body weight and food consumption were available for all but the high-dose group; due to the mortality in this group, only the initial body weight was available. Food consumption for this group was assumed to be equal to the intake measured in the next lower dose group (20,000 ppm group) for the purpose of these calculations.

dose groups of either sex. There was some evidence for an effect of 2,4-DCP treatment on body weight. In male mice exposed to 20,000 ppm, terminal body weight was 12% below that of controls ($p<0.01$ based on t-test conducted for this review). In females at this concentration, body weights were reduced by about 10-15% from control values during most of the study, but there was no difference from controls in terminal body weight due to a 10% decline in control weights during the final week; the authors did not suggest a cause for this decrease in control weights. Average food consumption (measured at weeks 7 and 13) was 67 to 77% of control values in mice of both sexes exposed to 10,000 ppm, and 44 to 57% of controls at 20,000 ppm; statistical comparison of the consumption rates was not presented. At 10,000 ppm and higher concentrations, mice of both sexes exhibited rough hair coats; the incidences were not reported. Histologic examination of the liver showed hepatocellular necrosis in male mice at all dose levels (0/10, 4/10, 4/10, 6/10, 10/10 at 0, 2500, 5000, 10,000, and 20,000 ppm). The increase was statistically significant ($p<0.05$) at all treatment levels; however, the severity was characterized as minimal at concentrations below 20,000 ppm. Syncytial alterations (multinucleated hepatocytes) were observed in all male mice exposed to 10,000 and 20,000 ppm 2,4-DCP, but not in exposed females, controls of either sex, or males exposed to 2500 or 5000 ppm. NTP (1989) noted renal tubular epithelial necrosis in eight male and three female mice at 40,000 ppm; all of these animals died in the first three weeks on study. The 2500 ppm concentration (782 mg/kg-day) is considered a minimal LOAEL based on mild hepatocellular necrosis in male mice; no NOAEL can be identified.

Exon et al. (1984; Exon and Koller, 1985) evaluated the immunotoxicity of 2,4-DCP in male and female Sprague-Dawley rats exposed prenatally or both pre- and postnatally. All animals were offspring (10/group) of dams exposed via drinking water to concentrations of 0, 3, 30 or 300 ppm 2,4-DCP (99% pure) from 3 weeks of age through breeding (at 90 days) and parturition (these were the same rats used in the reproductive study reported in the same publication; see below). Upon weaning, the prenatal-only groups were maintained untreated until 6 weeks of age, when immunocompetence was assessed. Dams of the pre- and postnatal groups were treated throughout the lactation period and, after weaning, offspring were given the treatment compound in the drinking water at the same concentrations until 13 weeks of age. Based on reported body weight and default water consumption values (U.S. EPA, 1988), the postnatal exposure concentrations correspond to doses of approximately 0.3, 3.0 and 30 mg/kg-day. The animals were weighed biweekly and observed daily for clinical signs. At 6 weeks and 13 weeks of age, respectively, the immunocompetence of groups treated prenatally and both pre- and postnatally was evaluated. Humoral and cell-mediated immune responses and macrophage function were assessed. Humoral immunity was quantified using ELISA assays for IgG antibodies to bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Cell-mediated immunity was measured using delayed-type hypersensitivity response to oxazolone (ear application) or BSA (footpad injection). Finally, macrophage function was assessed *in vitro*. Body and organ weights (liver, spleen and thymus) were recorded at sacrifice; complete necropsies were performed, as well as histopathologic evaluation of the liver, spleen and thymus.

Among the rats treated prenatally (and not postnatally), there was no effect on body weight when measured at 6 weeks of age; however, absolute spleen weights were significantly increased (29%, $p<0.05$) in rats treated at 300 ppm. Prenatal exposure alone did not significantly

alter any of the immune parameters assessed, and there were no significant histopathology findings.

Among rats treated at 300 ppm both pre- and postnatally, there was a significant increase (35%, $p \leq 0.05$) in anti-KLH antibody production compared with controls. Delayed-type hypersensitivity response, measured as the mean footpad swelling, was significantly reduced at both 30 and 300 ppm (40% and 43%, $p \leq 0.05$) (See Table 1). Absolute spleen and liver weights were significantly increased at the highest concentration (almost 2-fold higher spleen weight and 19% higher liver weight; $p \leq 0.05$); body and thymus weights were not affected. There were no histopathological differences among the groups. The LOAEL from this study is 30 ppm (3 mg/kg-day) based on decreased cell-mediated immunity (delayed-type hypersensitivity response); the NOAEL is 3 ppm or 0.3 mg/kg-day.

Table 1. Significant effects of prenatal and postnatal exposure to 2,4-DCP on immune parameters

2,4-DCP (ppm)	Anti-KLH antibody production (mean ± SE absorbance at 405 nm)	Delayed-type hypersensitivity (mean ± SE mm footpad swelling)
0	1.24 ± 0.10	1.10 ± 0.13
3	1.30 ± 0.10	0.85 ± 0.11
30	1.39 ± 0.10	0.67 ± 0.11 [a]
300	1.68 ± 0.08 [a]	0.63 ± 0.11 [a]

[a] Significantly different by analysis of variance and least-square means comparison.

Source: Exon et al., 1984; Exon and Koller, 1985.

Chronic Exposure — In the chronic NTP studies, F344/N rats and B6C3F1 mice (50 animals/sex/dose) were administered 2,4-DCP (>99% pure) in feed for 103 weeks (NTP, 1989). Dietary concentrations of 0, 5000 or 10,000 ppm were given to male rats and mice of both sexes; female rats were given concentrations of 0, 2500 or 5000 ppm. The authors estimated the doses of 2,4-DCP to be 210 or 440 mg/kg-day for male rats, 120 or 250 mg/kg-day for female rats, 800 or 1300 mg/kg-day for male mice and 430 or 820 mg/kg-day for female mice. Clinical observations were conducted twice daily, while body weight was measured weekly through week 13 and then monthly thereafter. Food consumption was measured monthly. After 103 weeks of dosing, all animals were sacrificed and necropsied. Histopathology of a comprehensive set of tissues (>40) was evaluated in control and high-dose animals; histopathology of low-dose animals was limited to the liver, nose, pituitary and thyroid of male rats; adrenal glands, lymph nodes, pancreas and spleen of female rats; liver, prostate, spleen and tarsal joints for male mice; and uterus for female mice.

Survival was not affected by treatment in rats of either sex, and there were no clinical signs of toxicity (NTP, 1989). Mean body weights in high-dose male and female rats were generally lower than those of controls (5-12%) beginning in week 3 (males) or week 31 (females), but statistical comparisons were not reported, nor were estimates of variability or individual body weight data that would permit statistical comparisons. Food consumption was significantly lower than controls (5-6%, $p<0.05$ based on t-tests conducted for this review) in males of both treatment groups and in high-dose females. Reductions in body weight predated differences in food consumption, indicating a toxic, rather than an organoleptic effect. The only nonneoplastic lesion that was significantly increased over controls was multifocal degeneration

of the nasal epithelium in male rats (25/45, 38/48, 42/46); the increases were significant at both doses ($p<0.05$). This study identifies a LOAEL of 210 mg/kg-day for nasal lesions in male rats; no NOAEL was identified.

There were no compound-related increases in the incidence of any neoplastic lesions in rats (NTP, 1989). The incidence of mononuclear cell leukemia was significantly decreased in dosed male rats relative to that in controls (control, 31/50; low dose, 17/50; high dose, 17/50).

In mice, treatment did not affect survival, nor were there any clinical signs of toxicity (NTP, 1989). Mean body weights of high-dose male mice and both dosed groups of female mice were generally lower than those of controls, although statistical comparisons were not provided. In high-dose males, body weight decrements occurred between weeks 25 and 86 and ranged from 3-9% in magnitude. In low-dose females, body weight decrements began in week 34 and ranged from 5-11%; in high-dose females, the reduction from control values increased over the course of the study and the mean terminal body weight was 17% below controls. Average food consumption was significantly lower than controls in high dose mice of both sexes (22% and 15%, for males and females, respectively; $p<0.05$ based on t-tests conducted for this review). However, body weight decrements preceded reductions in food consumption temporally, indicating that palatability of the diet was not an issue. A dose-related increase in the incidence of syncytial alteration of hepatocytes was observed in dosed male mice (11/50; 33/49; 42/48; $p<0.01$ at both doses based on Fisher's exact tests conducted for this review). This effect was also observed in the subchronic toxicity study with male mice, in which other evidence of liver toxicity (hepatocellular necrosis) was also observed. A LOAEL of 800 mg/kg-day was identified based on liver lesions (syncytial alteration of hepatocytes) in male mice and no NOAEL was identified.

As with rats, treatment did not result in a significantly increased incidence of any neoplastic lesion in mice (NTP, 1989). There was a marginally significant dose-related trend ($p=0.037$) in the incidence of squamous papilloma or carcinoma of the forestomach in male mice (0/50, 0/50, 3/50), but pairwise comparison did not indicate a significant increase at the high dose ($p=0.121$). The authors did not consider this increase to be treatment-related, as there was a negative trend for female mice and 2,4-DCP treatment did not increase the incidence of forestomach hyperplasia in the male mice. The incidence of malignant lymphomas was decreased in high-dose female mice (4/50) relative to controls (12/50). Under the conditions of these 2-year feeding studies, there was no evidence of carcinogenic activity for rats and mice fed 2,4-DCP.

In an oral carcinogenicity study, groups of Sprague-Dawley rats received both pre-and postnatal exposure to 2,4-DCP (99% pure) at concentrations of 0, 3, 30 or 300 ppm in drinking water (0, 0.45, 4.5 or 45 mg/kg-day[2]) for up to 24 months (Exon and Koller, 1985). Groups of 13 female rats were exposed from weaning through breeding at 90 days of age and until parturition. Offspring (groups of about 24/sex) were then given the test compound in drinking water from weaning until death or 24 months of age. Daily clinical observations were conducted, body weights of offspring were measured monthly and blood samples for hematology

[2] Based on default values for body weight and water consumption (U.S. EPA, 1988).

(erythrocyte and leukocyte counts, Hb, mean corpuscular volume [MCV] and packed-cell volume) were collected bimonthly. Necropsies were performed on tumor-bearing or moribund animals (and, presumably, survivors sacrificed at study termination), including histologic examination of the lung, heart, liver, spleen, kidney, adrenal, intestine, stomach, urinary bladder, brain, spinal cord, muscle and any tumors. The authors did not report any information on body weights or clinical signs of toxicity for the carcinogenicity study. When data from males and females exposed to 300 ppm 2,4-DCP for 14 months were combined, both erythrocyte count and Hb content were significantly increased (9% and 16%, respectively; $p \leq 0.05$). No other hematology data were reported or discussed. The toxicological significance of this finding is uncertain in the absence of data from other exposure levels and/or time periods; however, more pronounced hematological effects (bone marrow atrophy) were observed in female rats exposed to higher doses (750 mg/kg-day, NTP, 1989). Because this study was aimed at assessing the carcinogenicity of 2,4-DCP and no data on nonneoplastic findings (other than the single hematology measurements) were reported, effect levels were not derived for this study.

2,4-DCP administration had no effect on the incidence, latency or types of tumors relative to untreated controls (Exon and Koller, 1985). In a cocarcinogenicity study conducted simultaneously, tumor incidences in rats treated prenatally with the carcinogen ethylnitrosourea (ENU) and exposed to 2,4-DCP (either prenatally, postnatally or both) were not different from ENU-only treated rats. However, the authors noted that the group treated only with ENU had an unusually low incidence of tumors, potentially confounding the results of the cocarcinogenicity study with 2,4-DCP.

Kobayashi et al. (1972) evaluated the toxicity of 2,4-DCP in ICR mice fed the compound in the diet for 6 months. The study was published in Japanese and was not translated for this review; the summary contained herein is based on the English abstract and tables. The purity of the compound was not specified in the abstract. Groups of seven mice were fed concentrations of 0, 0.02%, 0.05%, 0.1% or 0.2% 2,4-DCP in the diet. From the available data, it appears that the high concentration group began treatment approximately 3 weeks later than the other groups; this is not discussed in the abstract. While the abstract does not specify the toxicological endpoints examined, the tables indicate that hematology (erythrocyte and leukocyte count), liver function (AST or ALT) and organ weights (liver, kidney, spleen and heart) were assessed in all treatment groups and histopathology evaluations (liver, kidney, spleen, heart and adrenal glands) were performed on controls and animals in the 0.1% and 0.2% groups.

Reuber (1983) reviewed all available studies related to the carcinogenicity of the phenoxy herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) and its primary metabolite, 2,4-dichlorophenol (2,4-DCP). This report indicated that while 2,4-D was carcinogenic to both male and female rats, 2,4-DCP only demonstrated promoter activity in mouse skin cancer studies.

Based on measured body weights and food consumption rates, the authors estimated the doses to be 0, 45, 100 and 230 mg/kg-day. The authors reported that there were no effects on behavior. Both the abstract and the tabulated data on body weight, food consumption, hematology, liver function and organ weights (liver, kidney) indicated no effect of 2,4-DCP treatment. The authors reported that the histologic examinations showed “slight unfavorable” effects on the liver (the table reports these as “small round cell infiltration, swelling of hepatic

cells, unequal size of hepatic cells and dark cells") at the highest dose; however, the numbers of affected animals were small (1 or 2 in a group of 7), so the toxicological significance is difficult to assess. The authors considered the 100 mg/kg-day dose to be a NOEL in mice. However, due to the small numbers of animals used in this study and the lack of a full translation, it was not considered appropriate to assign effect levels based on these data.

Reproductive/Developmental Studies — Aoyama et al. (2005) conducted a two-generation reproductive toxicity study in Wistar-Hannover rats exposed to 2,4-DCP via the diet. Groups of 24 rats/sex/group (aged 5 weeks) were given 2,4-DCP (99.7% pure) at concentrations of 0, 500, 2000 or 8000 ppm for 10 premating weeks and during mating, gestation and lactation. Pregnant dams were allowed to give birth and on postnatal day (PND) 4, the litters were culled to 8 pups (4/sex when possible). On PND 21, groups of 24 male and female weanlings (1 male and 1 female from each litter) were selected to become F1 parents. F0 parents and any weanlings not selected to be F1 parents were necropsied at this time. F1 parents were treated in the same manner as the F0 parents with dietary 2,4-DCP during 10 premating weeks and through lactation of the F2 pups. Upon weaning of the F2 generation, F1 parents and F2 pups were sacrificed and necropsied.

Clinical observations of parental animals were conducted daily and body weights and food consumption measured weekly (Aoyama et al., 2005). Female estrous cyclicity was evaluated by vaginal smear for 2 weeks prior to mating. Upon parturition, fertility and gestation parameters were recorded and the number and sex of live pups noted. Pups were weighed on PND 0, 4, 7, 14 and 21. Developmental milestones (pinna unfolding, tooth eruption, eye opening) were recorded in both generations. In addition, age at preputial or vaginal opening was evaluated in F1 pups selected to be parents, while anogenital distance on PND 4 was recorded in F2 pups. At necropsy (after weaning of pups for parental animals and at weaning for F2 pups), the number of uterine implantation sites was noted in female parents and sperm count and motility were recorded in male parents. The following organs were examined histologically in parental animals: liver, kidneys, pituitary, reproductive organs of both sexes and mammary glands. In F2 pups, the brain, thymus, spleen and uterus were weighed, but not examined histologically. Levels of pituitary and ovarian hormones (FSH, LH, prolactin, 17ß-estradiol and progesterone) were measured in F1 parent females upon necropsy.

Using mean body weight and food consumption values, the authors estimated doses of 33.4, 134 and 543 mg/kg-day for males and 49.1, 194 and 768 mg/kg for females (Aoyama et al., 2005). Clinical signs of toxicity (soiled fur in the abdominal/genital regions) were apparent in the highest dose group. At the highest dose, food consumption and body weight were significantly ($p \leq 0.01$) lower in both males and females of the F0 generation and in parental females of the F1 generation throughout most of premating, gestation and lactation (data presented graphically). Females exposed at 2000 ppm also exhibited occasional statistically significant (p-value and data not reported) reductions in food consumption and/or body weight (during premating in the F0 generation and during gestation in the F1 generation).

The mean number of implantation sites was slightly decreased in a dose-dependent fashion in both F0 and F1 parents, but was statistically significant ($p \leq 0.05$) only at the high dose in the F1 generation (10.2 vs. 12.7 in controls; Aoyama et al., 2005). In addition, the number of

live pups appeared to decrease with dose in both generations, but there were no statistically significant reductions. The age at preputial separation was significantly ($p \leq 0.05$) longer (42.2 days vs. 41.2 days in controls) in high-dose parental males of the F1 generation; however, the authors attributed this effect to reduced body weight. In contrast, despite lower body weight in high-dose females (F1), the age at vaginal opening was accelerated, albeit nonsignificantly (31.5 days vs. 32.2 days in controls). Neither sperm parameters nor female hormone concentrations were affected by treatment. Among high-dose parents, significant increases in relative organ weight (kidneys, testes) occurred in the absence of absolute organ weight changes and were attributable to decreased body weight. No other organ weight changes occurred in a dose-dependent manner and/or across generations.

Birth weight of pups was not affected by treatment (Aoyama et al., 2005). Pups of high-dose parents of both generations had reduced body weights (compared with controls) beginning on PND 7 and continuing through weaning. As dams treated at this dose (both generations) had significantly reduced food consumption and body weight, the reductions in pup weight are not unexpected. At the highest dose, eye opening was significantly ($p \leq 0.01$) delayed in pups of both generations and both sexes (50.5 to 65.3% of pups with eyes open on PND 14 vs. 89.1 to 94.6% of controls). Other developmental parameters were not affected by treatment. Upon necropsy of weanlings, there were significant reductions in absolute weights of brain, thymus and spleen in the high-dose group; however, these were attributable to reduced body weight. In contrast, the uterus weight of both F1 and F2 females was significantly increased at the high dose (42% and 20%, respectively; $p \leq 0.01$) and at the mid-dose in F1 females (25%, $p \leq 0.05$). Histologic examination of selected uteri of high-dose weanlings in the F2 generation indicated increased epithelial cell height (7/10 vs. 1/10 controls).

The authors indicated that the NOAEL for parental toxicity was 500 ppm and called the 2000 ppm concentration the "minimum toxic dose" based on reduced food consumption and body weight in parental females. Palatability of the treatment diet was not likely the cause of reduced weight. There were no reductions in food consumption or body weight in F0 males, indicating that this group consumed the treatment compound readily. Further, body weight reductions in high-dose F1 females preceded reductions in food consumption, indicating a toxic effect rather than an organoleptic effect. Body weight reductions have been reported in other studies (NTP, 1989), including a gavage study (Rodwell et al., 1989). Thus, the 2000 ppm concentration (194 mg/kg-day in females) is considered a minimal LOAEL based on reduced body weight and the 500 ppm concentration (49.1 mg/kg-day in females) is the NOAEL for parental toxicity.

For reproductive endpoints, the authors identified the 8000 ppm concentration (768 mg/kg-day in females) as a toxic dose based on reduced number of implantation sites in F1 parental females, increased uterine weight in F1 and F2 weanlings and accelerated sexual maturation of F1 females. In addition, eye opening was significantly delayed at this dose. The 2000 ppm concentration (194 mg/kg-day in females) is a NOAEL for reproductive effects.

Exon et al. (1984; Exon and Koller, 1985) evaluated a limited number of reproductive parameters in a study using female Sprague-Dawley rats exposed to 2,4-DCP (99% pure) from 3 weeks of age, through breeding with untreated males and until parturition. This information was

collected in conjunction with the immunotoxicity study on the progeny of exposed rats (see above). Groups of 13 rats were exposed via drinking water to concentrations of 3, 30 or 300 ppm 2,4-DCP (estimated to result in doses of 0.45, 4.5 and 45 mg/kg-day[3]). Percent conception, litter size, percent stillborn, birth and weaning weight and survival to weaning were evaluated. The authors reported that there were no treatment-related effects on the dams. Although the text indicated that the percent stillborn tended to be greater in the treatment groups, the increases were not statistically significant (2% in the high- and mid-concentration groups, 1% in the low concentration group and 0 in controls). The average litter size was smaller in the high-concentration group (6.3±1.6) compared with controls (9.8±1.3), but the difference was not statistically significant[4]. Other parameters were not affected by treatment. Due to the limited number of parameters assessed in this study, effect levels were not identified.

A two-generation rat reproductive study was conducted to investigate potential endocrine-mediated effects. One of the herbicides, 2,4-dichlorophenol, was administered at 0, 500, 2000 and 8000 ppm in the diet. Increased uterine weights were observed in both F1 and F2 females. A reduced number of implantation sites and reduced live births in F1 parental animals were observed at 2000 ppm and higher exposure levels (Yamasaki et el., 2005).

To understand the structural basis for estrogenic activity, Tarasaka et al. (2006) performed DNA-micro array assay of several structurally similar chemicals, including 2,4-dichlorophenol. This assay demonstrated the estrogenic activity of 2,4-dichlorophenol by down regulating enzymes and signaling pathway compared to chemicals with high levels of estrogenic activity.

Using prostate cancer cell lines, Kim et al. (2005) evaluated the endocrine disrupting activity of 2,4-dichlorophenoxyacetic acid and 2,4-dichlorophenol. These chemicals did not show any androgenic activity. However, co-exposure with 5alpha-dihydroxytestosterone synergistic androgenic activity was demonstrated in this assay.

Rodwell et al. (1989; Dow Chemical Co., 1983) evaluated the developmental toxicity of 2,4-DCP in F344 rats. Groups of 34 sperm-positive female rats were given 2,4-DCP (99.2% pure, in corn oil) via gavage on gestation days (GD) 6-15. Doses of 0, 200, 375 or 750 mg/kg-day were administered. Maternal body weights were recorded on GD 0, 6, 10, 12, 15 and 20. On GD 20, dams were sacrificed by carbon dioxide and subjected to caesarean section. The uterus was weighed and examined for number and location of viable and nonviable fetuses and early and late resorptions. Number and location of *corpora lutea* were recorded. Fetuses were weighed, measured, sexed and examined externally; half were then prepared for soft tissue examination and the remainder prepared for skeletal examination.

Four dams in the high-dose group died during treatment (Rodwell et al., 1989; Dow Chemical Co., 1983). Clinical observations in the high-dose group included red staining around the eyes, nares and mouth; abdominal alopecia; and respiratory rales. While incidences were not

[3] Based on default values for body weight and water consumption (U.S. EPA, 1988).
[4] Exon and Koller (1985) reported that the decrease in litter size was statistically significant at $p \leq 0.10$; Exon et al. (1984) reported that it was not statistically significant. The latter interpretation was accepted here given the relatively high critical value (0.10) used to assess significance in the 1985 paper.

reported, the authors indicated that these were observed in a majority of animals at this dose and in some animals exposed to 375 mg/kg-day. A few high-dose rats also displayed ataxia, prostration and reduced activity. In addition, yellow staining of fur in the urogenital area occurred in all treated groups (incidence not provided, but reported to increase with dose). Maternal body weight gain during treatment was significantly ($p<0.05$) lower than control weight gain in all dose groups (82%, 77%, and 32% of controls during GD 6-15 at the low, mid-, and high doses); further, the body weight decrements persisted after exposure ceased, although the decrease was statistically significant only at the high dose. There were no significant differences in reproductive or teratogenic parameters. Significantly ($p<0.05$) increased incidences of unossified sternebrae (4/22 litters vs. 0/27 litters in controls) and delayed ossification of vertebral arches (6/22 litters vs. 0/27 litters in controls) were observed in the high-dose group. The high dose in this study was a Frank Effect Level (FEL) due to 4 maternal deaths. The LOAEL for maternal toxicity was 200 mg/kg-day, based on clinical signs and decreased body weight gain during treatment; no NOAEL can be identified for maternal toxicity. Developmental effects (increased skeletal variations) were observed at the maternal FEL of 750 mg/kg-day, but not at lower doses; thus, the NOAEL for developmental toxicity was 375 mg/kg-day.

Inhalation Exposure

The literature search identified no studies regarding toxicity of 2,4-DCP in animals following inhalation exposure. Similarly, the available reviews (U.S. EPA, 1987a,b; ATSDR, 1999; IARC, 1986, 1999; WHO, 1989) did not identify any inhalation toxicity studies of this compound.

Other Studies

Acute Toxicity — Borzelleca et al. (1985a,b) reported oral LD50 values of 1276 mg/kg and 1352 mg/kg for male and female CD1-ICR mice observed for up to 14 days. Kobiyashi et al. (1972) calculated oral LD50 values of 1600 mg/kg in ICR mice (same value for males and females) and 3670 and 4500 mg/kg in male and female Sprague-Dawley rats (respectively) observed for up to 10 days.

Dermal Carcinogenicity Studies — Boutwell and Bosch (1959) examined the ability of 2,4-DCP to act as a complete carcinogen on the skin of mice and to promote skin tumors following a single initiating dose of dimethylbenzanthracene (DMBA). In the study for complete carcinogenesis, a group of 23 female Sutter mice (2-3 months of age) was treated with a topical application of 25 µL of 20% 2,4-DCP in benzene applied twice weekly to the back of each mouse for 24 weeks. Of the 23 mice, 16 (70%) survived to 24 weeks, at which time 75% of the survivors (12 mice) had papillomas and 6% (1 mouse) had a carcinoma. The average number of papillomas per mouse was 1.62. At 39 weeks, 62% of surviving mice (number not reported) had carcinomas. There was no control group maintained concurrently in this experiment. The absence of a similarly-treated concurrent control group, along with the significant mortality (30%) in this short-duration study, limits the usefulness of these data.

In the promotion experiment, a group of 33 female Sutter mice was treated with an initial topical application of 75 µL of 0.3% DMBA in benzene, followed by 25 µL of 20% 2,4-DCP in benzene applied twice weekly to the back of each mouse for 15 weeks (Boutwell and Bosch, 1959). At 15 weeks, 27 of 33 (82%) 2,4-DCP-treated mice survived, compared to 15 of 20 (75%) in the control group. Based on information in the publication, the control group was treated with an initiating dose of DMBA; it is not clear whether the controls received applications of vehicle (benzene) on the promotion schedule[5]. At the end of treatment, the incidence of papillomas was significantly increased ($p<0.01$) in 2,4-DCP-treated surviving mice (13/27), compared with controls (1/15). The average number of papillomas per mouse was 1.07 vs. 0.07 in controls. Three of 27 (11%) 2,4-DCP-treated survivors had carcinomas, compared to no carcinomas in the initiator only group; however this difference was not statistically significant ($p>0.05$).

U.S. EPA (1980) criticized several aspects of this study, including the failure to histologically confirm tumor types and the use of creosote-coated wooden cages to house the animals. The use of creosote-treated cages could not be confirmed; the publication indicates that "screen-bottomed metal cages" were used. In addition, U.S. EPA (1980) noted that the high concentration of 2,4-DCP (20% in benzene) applied to the skin may have caused physical abrasion of the skin. 2,4-DCP is known to be corrosive to the skin (HSDB, 2006) and this irritant property may have enhanced the papillomatous response in both studies. It is also important to note that there was significant mortality in the control group of the promotion study (25%) despite the short duration of the study (15 weeks). The reason for this high rate of mortality or that of the 2,4-DCP treated group in the complete carcinogenicity study (30%), was not discussed by the authors.

Genotoxicity — Genotoxicity testing of 2,4-DCP has generally given negative results. Positive results in clastogenicity testing have often been associated with cell toxicity. 2,4-DCP produced no increases in revertant colonies in *Salmonella typhimurium* strains TA98, TA100 or TA1537 with or without exogenous metabolic activation (Haworth et al., 1983; Rasanen et al., 1977). The mutagenic effect of 2,4-DCP in *Salmonella typhimurium* strain TA1535 was initially considered to be equivocal in the presence of hamster liver S9 metabolic activation (Haworth et al., 1983). However, a reevaluation of the data resulted in a determination that the response was negative (Zeiger, 1990). 2,4-DCP significantly increased trifluorothymidine (Tft) resistance in the mouse L5178Y assay at concentrations of 30-60 µg/mL when tested without metabolic activation (Myhr et al., 1990). 2,4-DCP was cytotoxic to V79 Chinese hamster cells, but did not induce 6-thioguanine-resistant mutants when tested at concentrations up to 50 µg/mL without exogenous metabolic activation (Jansson and Jansson, 1986). In a cell-mediated test (wherein metabolic activation was provided by co-cultured cells), 2,4-DCP was weakly mutagenic at concentrations that were also cytotoxic to V79 cells (cell survival 41-54% of controls; Fiskesjo, 1988).

In cultured CHO cells, 2,4-DCP did not induce chromosomal aberrations at concentrations up to 75 µg/mL (0.51 mM) without S9 (8-hour treatment) and at up to 150 µg/mL

[5] In other experiments using DMBA followed by promotion testing of agents dissolved in benzene, the control is reported as a "benzene control"; in this experiment, it is not.

(1.02 mM) with S9 (2-hour treatment) (Anderson et al., 1990). In another CHO cell assay, chromosomal aberrations developed in a significant percentage of cells both with and without activation (Hilliard et al., 1998). Aberrations were observed in 14% of cells following a 3-hour treatment at 1.4 mM without S9 and in 14.5% cells treated at 0.6 mM with S9, compared with 1.5% of control cells (Hilliard et al., 1998). Cell survival was reduced in both of these cases (27% and 54% of controls, respectively), raising the possibility that the aberrations were related to toxicity (Hilliard et al., 1998). Testing of human TK6 lymphoblasts for chromosomal aberrations resulted in an equivocal (nonsignificant) increase (5% of cells with aberrations, compared with 0% in controls) for 2,4-DCP at 0.8 mM, a concentration that gave 59% survival compared with controls. 2,4-DCP increased the frequency of sister chromatid exchanges (SCEs) both in the presence and absence of S9 (Anderson et al., 1990).

The cytogenic effect of 2,4-dichlorophenol was studied in bone marrow, germ cells and spermhead abnormalities in mice treated intraperitoneally at 1/10, 1/5, ½ dose levels (Amer and Aly, 2001). This report demonstrated weaker genotoxic effects as indicated by lower percentage of induced chromosomal aberrations and spermhead abnormalities.

2,4-DCP induced error-prone DNA repair (*umu*-test) in *S. typhimurium* cells when tested without metabolic activation (Ono et al., 1992). In an *in vitro* alkaline elution/rat hepatocyte genotoxicity assay, 2,4-DCP produced evidence of DNA damage; however, cytotoxicity tests showed significant toxicity at concentrations resulting in DNA damage and the authors suggested that DNA effects likely resulted from activation of degradative endonucleases in dead or dying cells (Storer et al., 1996). 2,4-DCP did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes at a concentration of 50 nmol/mL (Probst et al., 1981). In a prophage-induction assay, 2,4-DCP did not induce DNA damage in *E. coli* at doses up to 480 μmol (DeMarini et al., 1990). 2,4-DCP produced negative results in an *in vivo-in vitro* mouse hepatocyte replicative DNA synthesis (RDS) test (Miyagawa et al., 1995).

Genotoxicity testing of 2,4-DCP is complicated by the fact that this compound uncouples oxidative phosphorylation, leading to depletion of cellular energy supplies. Mitsuda et al. (1963) reported that a concentration of 42 μM 2,4-DCP caused 50% inhibition of ATP production in rat liver mitochondria *in vitro*; in fact, 2,4-DCP was the most potent of the mono- and dichlorophenols tested in this study. Disturbances in energy production may be responsible for the cellular toxicity observed in genotoxicity assays, many of which were conducted at high concentrations. Cell toxicity can lead to false-positive findings, especially in assays for clastogenicity, because DNA damage commonly occurs in apoptotic and necrotic cells (Storer et al., 1996; Hilliard et al., 1998).

DERIVATION OF A PROVISIONAL SUBCHRONIC ORAL RfD VALUE FOR 2,4-DICHLOROPHENOL

A chronic oral RfD of 0.003 mg/kg-day based on an immunotoxicity study (Exon et al., 1984) is available on IRIS. Several oral toxicity studies, including the immunotoxicity study, are available for use in deriving a provisional subchronic oral RfD for 2,4-DCP. Table 2 summarizes the findings of those studies in which a NOAEL and/or LOAEL was identified. As

Table 2. Summary of Oral Noncancer Dose-Response Information

Species	Sex	Doses (mg/kg-day)	Exposure Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Responses	Comments	Reference
Chronic Studies								
Rats	M/F	0, 210, 440 (M) 0, 120, 250 (F)	103 weeks	NA	210	Multifocal degeneration of nasal epithelium		NTP, 1989
Mice	M/F	0, 800, 1300 (M) 0, 430, 820 (F)	103 weeks	NA	800	Syncytial alteration of hepatocytes in males		NTP, 1989
Subchronic Studies								
Rats	M/F	0, 160, 310, 675, 1373 (M) 0, 182, 338, 750, 1376, 2795 (F)	13 weeks	338	750	Bone marrow atrophy in females		NTP, 1989
Mice	M/F	0, 782, 1533, 1627, 2960, 6805 (M) 0, 973, 2438, 3305, 3913, 8911 (F)	13 weeks	NA	782	Mild hepatocellular necrosis		NTP, 1989
Rats	M/F	0, 0.3, 3, 30	Pre- and postnatal	0.3	3	Decreased cell-mediated immunity	Immunotoxicity study. Exposure prenatally, through lactation and via drinking water from weaning until 13 weeks of age	Exon and Koller, 1985
Reproductive/Developmental Studies								
Rats	F	0, 200, 375, 750	GD 6-15	NA (maternal) 375 (develop-mental)	200 (maternal) 750 (develop-mental)	Maternal toxicity: clinical signs, reduced body weight gain Developmental effects: increased incidence of skeletal variations		Rodwell et al., 1989; Dow Chemical Co., 1983

Table 2. Summary of Oral Noncancer Dose-Response Information

Species	Sex	Doses (mg/kg-day)	Exposure Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Responses	Comments	Reference
Rats	M/F	0, 33.4, 134, 543 (M) 0, 49.1, 194, 768 (F)	10 weeks premating, through gestation and lactation	49.1 (parental) 194 (reproductive)	194 (parental) 768 (reproductive)	Parental toxicity: transient reductions in body weight of dams Reproductive effects: reduced number implantation sites (F1 dams), delayed eye opening and effects on uterus in offspring	Two-generation reproductive toxicity study	Aoyama et al., 2005

the table indicates, the LOAEL for immunotoxicity (3 mg/kg-day; Exon et al., 1984) is much lower (almost 2 orders of magnitude) than LOAELs for other endpoints. This study was thus selected as the basis for the subchronic p-RfD. The critical effect in this study is a decrease in cell-mediated immunity, as measured by decreased footpad swelling. The data selected for modeling are shown in Table 3. Models for continuous variables in U.S. EPA's Benchmark Dose Software (BMDS) were fit to the cell-mediated immunity data in accordance with U.S. EPA (2000) methodology. A default benchmark response (BMR) of one standard deviation from the control mean was used. Appendix A contains details of the modeling and a plot of the best fitting model.

Table 3. Cell-mediated Immunity Data as Modeled

2,4-DCP (ppm)	2,4-DCP (mg/kg-day)	No. of rats	Delayed-type hypersensitivity (mm footpad swelling)		
			Mean	SE	SD
0	0	10	1.10	0.13	0.41
3	0.3	10	0.85	0.11	0.35
30	3.0	10	0.67	0.11	0.35
300	30	10	0.63	0.11	0.35

Source: Exon et al. (1984; Exon and Koller, 1985)

The test for homogenous variance indicated that the homogenous variance model provided adequate fit to the variance data. Using the homogenous variance model, the linear model did not provide adequate fit to the means, so the remaining models were applied; however, none provided adequate fit. In order to try to achieve model fit, the high dose group was dropped from the analysis. Using the reduced data set, the homogenous variance model again provided adequate fit to the variance data, and the linear model provided adequate fit to the means. BMD and BMDL predictions from the modeling of the reduced data set were 3.21 and 1.84 mg/kg-day, respectively. The BMDL from this study (2 mg/kg-day) was thus selected as the point-of-departure (POD) for derivation of the subchronic p-RfD.

The **subchronic p-RfD of 0.02 mg/kg-day** is calculated as the BMDL of 2 mg/kg-day divided by an uncertainty factor of 100, as shown below:

Subchronic p-RfD = BMDL/UF
= 2 mg/kg-d / 100
= 0.02 mg/kg-day

An interspecies uncertainty factor of 10 was applied and another 10-fold uncertainty factor was used for protection of sensitive individuals. A factor of 1 for duration was applied, as the exposure included prenatal and lactational exposure, followed by exposure via drinking water to 13 weeks of age. Since a BMDL was used as the POD, no adjustment for use of a LOAEL was necessary. No database uncertainty factor was used; the toxicological database for 2,4-DCP contains chronic studies in two species, several subchronic studies in rats and mice, two developmental toxicity studies in rats, and a multigeneration reproductive toxicity study in rats. The database lacks a neurotoxicity study; however, existing studies suggest that neurotoxic effects occur only at high doses.

Confidence in the principal study (Exon et al., 1984) is medium because, despite the investigation of sensitive endpoints, the sample sizes were relatively small (10 per dose). Confidence in the database is high because the database includes well-conducted chronic studies in two species, several subchronic studies in rats and mice, two developmental toxicity studies in rats, and a multigeneration reproductive toxicity study in rats. Medium to high confidence in the subchronic p-RfD follows.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION p-RfC VALUES FOR 2,4-DICHLOROPHENOL

There are no inhalation studies available for use in developing subchronic and/or chronic provisional RfCs (p-RfC) for 2,4-DCP.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 2,4-DICHLOROPHENOL

Weight-of-Evidence Classification

Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), 2,4-DCP is *not likely to be carcinogenic to humans via oral exposure.* There is *inadequate information to assess the carcinogenic potential* of 2,4-DCP to humans via inhalation exposure. There are no human data addressing the potential carcinogenicity of 2,4-DCP alone, either via oral or inhalation exposure. 2,4-DCP tested negative in adequate 2-year NTP dietary bioassays using both rats and mice. In addition, a second adequate chronic study in rats found no increase in tumor formation with chronic 2,4-DCP exposure (Exon and Koller, 1985). 2,4-DCP has not been tested for carcinogenicity via inhalation exposure. In an old publication where dermally-applied 2,4-DCP was tested both for complete carcinogenicity and as a promoter, an increased incidence of papillomas was observed (Boutwell and Bosch, 1959); however, there are a number of limitations that call into question these results, including: lack of control in the complete carcinogenicity study, high mortality in the control group for the promotion study and use of a potentially corrosive concentration of 2,4-DCP in the skin applications. Genotoxicity testing of 2,4-DCP has largely given negative responses; instances where positive responses were reported have often been associated with cytotoxicity.

Quantitative Estimates of Carcinogenic Risk

There are no appropriate human or animal data from which to derive an oral slope factor or inhalation unit risk for 2,4-DCP.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 2006. Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. ACGIH, Cincinnati, OH.

Amer, S.M. and F.A.E. Aly. 2001 Genotoxic effect of 2,4-dichlorophenoxy acetic acid and its metabolite 2,4-dichlorophenol in mouse. Mutat. Res. 494:1-12.

Anderson, B.E., E. Zeigler, M.D. Shelby et al. 1990. Chromosome aberration and sister chromatid exchange test results with 42 chemicals. Environ. Mol. Mutagen. 16:55-137.

Aoyama, H., H. Hojo, K.L. Takahashi et al. 2005. A two-generation reproductive toxicity study of 2,4-dichlorophenol in rats. J. Toxicol. Sci. 30(spec. iss.):59-78.

ATSDR (Agency for Toxic Substances and Disease Registry). 1999. Toxicological Profile for Chlorophenols. U.S. Department of Health and Human Services, Public Health Service. Atlanta, GA. Available at http://www.atsdr.cdc.gov/toxprofiles/tp107.html.

Axelson, O., L. Sundell, K. Andersson et al. 1980. Herbicide exposure and tumor mortality. Scand. J. Work Environ. Health. 6:73-79. (Cited in Lynge, 1987)

Bleiberg, J., M. Wallen, R. Brodkin et al. 1964. Industrially acquired porphyria. Arch. Dermatol. 89:793-797. (Cited in ATSDR, 1999).

Borzelleca, J.F., J.R. Hayes, L.W. Condie et al. 1985a. Acute and subchronic toxicity of 2,4-dichlorophenol in CD-1 mice. Fund. Appl. Toxicol. 5:478-486.

Borzelleca, J.F., J.R. Hayes, L.W. Condie et al. 1985b. Acute toxicity of monochlorophenols, dichlorophenols and pentachlorophenol in the mouse. Toxicol. Lett. 29(1):39-42.

Boutwell, R.K. and D.K. Bosch. 1959. The tumor-promoting action of phenol and related compounds for mouse skin. Cancer Res. 19:413-424.

Bueno de Mesquita, H.B., G. Doornbos, D. Van der Kuip et al. 1993. Occupational exposure to phenoxy herbicides and chlorophenols and cancer mortality in the Netherlands. Am. J. Ind. Med. 23:289-300.

DeMarini, D.M., H.G. Brooks and D.G. Parkes, Jr. 1990. Induction of prophage *lambda* by chlorophenols. Environ. Mol. Mutagen. 15:1-9.

Dow Chemical Co. 1983. Initial submission: Teratology study with 2,4-dichlorophenol in Fischer 344 rats with cover letter dated 06/23/92. TSCATS submission. Fiche No.OTS540423.

Eriksson, M., L. Hardell, N.O. Berg et al. 1981. Soft tissue sarcomas and exposure to chemical substances: A case-referent study. Br. J. Ind. Med. 38:27-33.

Exon J.H., G.M. Henningsen, C.A. Osborne and L.D. Koller. 1984. Toxicologic, pathologic, and immunotoxic effects of 2,4-dichlorophenol in rats. J. Toxicol. Environ. Health. 14:723-730.

Exon J.H. and L.D. Koller. 1985. Toxicity of 2-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol. Water Chlorination, Vol. 5 Chemistry, Environmental Impact and Health Effects: Proceedings of the 5th Conference on Water Chlorination. Environmental Impact and Health Effects, Williamsburg, VA June 3-8, 1984. pp. 307-330.

Fiskesjo, G. 1988. 2,4-Dichlorophenol and MCPA in a V79 test. Scand. Cell Toxicol. Congress. 10:245-250.

Gingell, R., J. O'Donoghue, R.J. Stabb et al. 2001. Phenol and phenolics ethers. In: Patty's Industrial Hygiene and Toxicology. 2006 Online Edition. Bingham, E., B. Cohrssen, and C.H. Powell, Eds. John Wiley and Sons, New York.

Hardell, L. 1981. On the relation of soft tissue sarcoma, malignant lymphoma and colon cancer to phenoxy acids, chlorophenols and other agents. Scand. J. Work Environ. Health. 7:119-130.

Hardell, L. and A. Sandstrom. 1979. Case-controls study: Soft tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br. J. Cancer. 39:711-717.

Hardell, L., M. Eriksson, P. Lenner et al. 1981. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids. A case-control study. Br. J. Cancer. 43:169-176.

Hardell, L., B. Johansson and O. Axelson. 1982. Epidemiological study of nasal and nasopharyngeal cancer and their relation to phenoxy acid or chlorophenol exposure. Am. J. Ind. Med. 3:247-257.

Haworth, S., T. Lawlor, K. Mortelmans et al. 1983. *Salmonella* mutagenicity test results for 250 chemicals. Environ. Mutagen. 1:3-142.

Hilliard, C.A., M.J. Armstrong, C.I. Bradt et al. 1998. Chromosomal aberrations *in vitro* related to cytotoxicity of nonmutagenic chemicals and metabolic poisons. Environ. Mol. Mutagen. 31:316-326.

Hogsted, C. and B. Westerlund. 1980. Cohort study of mortality among forest workers with and without exposure for phenoxy herbicides. Laektidn. 77:1828-1830. (Cited in Lynge, 1987)

HSDB. 2006. 2,4-Dichlorophenol. Hazardous Substances Data Bank. National Library of Medicine. Available at http://toxnet.nlm.nih.gov.

IARC (International Agency for Research on Cancer). 1986. Occupational exposures to chlorophenols. IARC monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 41, p. 319-356.

IARC (International Agency for Research on Cancer). 1999. Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide. IARC monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 71, p. 769.

Jansson, K. and V. Jansson. 1986. Inability of chlorophenols to induce 6-thioguanine- resistant mutants in V79 chinese hamster cells. Mutat. Res. 171:165-168.

Kim, H., Y.Z. Payk and M. Dong. 2005. Effect of 2,4-D and DCP on DHT-induced androgenic action in human prostate cancer cells. Toxicol. Sci. 88:52-59.

Kintz P., A. Tracqui, and P. Mangin. 1992. Accidental death caused by the absorption of 2,4-dichlorophenol through the skin. Arch Toxicol. 66:298-299.

Kobayashi, S., S. Toida, H. Kawamura, H.S. Chang, T. Fukuda and K. Kawaguchi. 1972. Chronic toxicity of 2,4-dichlorophenol in mice: A simple design for the toxicity of residual metabolites of pesticides. J. Med. Soc. Toho Japan. 19(3-4):356-362.

Kogevinas M., R. Saracci, P.A. Bertazzi et al. 1992. Cancer mortality from soft-tissue sarcoma and malignant lymphomas in an international cohort of workers exposed to chlorophenoxy herbicides and chlorophenols. Chemosphere. 25(7-10):1071-1076.

Kogevinas, M., R. Saracci, R. Winkelmann et al. 1993. Cancer incidence and mortality in women occupationally exposed to chlorophenoxy herbicides, chlorophenols and dioxins. Cancer Causes Control. 4:547-553.

Lynge, E. 1985. A follow-up study of cancer incidence among workers in manufacture of phenoxy herbeicides in Denmark. Br. J. Cancer. 52(2):259-270.

Lynge, E. 1987. Background and design of a Danish cohort study of workers in phenoxy herbicide manufacture. Am. J. Ind. Med. 11:427-437.

Mitsuda H., K. Murakami and F. Kawai. 1963. Effect of chlorophenol analogues on the oxidative phosphorylation in rat liver mitochondria. Agr. Biol. Chem. 27(5):366-372.

Miyagawa, M., H. Takasawa, A. Sugiyama et al. 1995. The *in vivo-in vitro* replicative DNA synthesis (RDS) test with hepatocytes prepared from male B6C3F1 mice as an early prediction assay for putative nongenotoxic (Ames-negative) mouse hepatocarcinogens. Mutat. Res. 343:157-183.

MMWR (Morbidity and Mortality Weekly Report). 2000. Occupational fatalities associated with 2,4-dichlorophenol (2,4-DCP) exposure, 1980-1998. MMWR 49(23):516-518.

Myhr, B., D. McGregor, L. Bowers et al. 1990. L5178Y mouse lymphoma cell mutation assay results with 41 compounds. Environ. Mol. Mutagen. 16:138-167.

NIOSH (National Institute for Occupational Safety and Health). 2006. NIOSH Pocket Guide to Chemical Hazards. Available at http://www.cdc.gov/niosh/npg/npgdcas.html.

NTP (National Toxicology Program). 1989. Toxicology and Carcinogenesis Studies of 2,4-Dichlorophenol (CAS No. 120-83-2) in F344/N Rats and B6C3F1 Mice (Feed Studies). June 1989. NTIS PB90-106170/AS. TR-353.

NTP (National Toxicology Program). 2006. 11th Report on Carcinogens. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. Available at http://ntp-server.niehs.nih.gov/.

Ono Y., I. Somiya and T. Kawaguchi. 1992. Genotoxic evaluation on aromatic organochlorine compounds by using *umu* test. Water Sci. Tech. 26(1-2):61-19.

OSHA (Occupational Safety and Health Administration). 2006. OSHA Regulations. Available at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table= STANDARDS&p_id=9992.

Poland, A.P., D. Smith, G. Metter et al. 1971. A health survey of workers in a 2,4-D and 2,4,5-T plant. Arch. Environ. Health. 22:316-327.

Probst, G.S., R.E. McMahon, L.E. Hill et al. 1981. Chemically-induced unscheduled DNA synthesis in rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds. Environ. Mutagen. 3:11-32.

Rasanen L., M.L. Hattula and A.U. Arstila. 1977. The mutagenicity of MCPA and its soil metabolites, chlorinated phenols, catechols and some widely used slimicides in Finland. Bull. Environ. Contam. Toxicol. 18:565.

Reuber, M.D. 1983. Carcinogenicity and toxicity of 2,4-dichlorophenoxy acetic acid. Sci. Total Environ. 31:203-218.

Riihimaki, V., A. Sisko and S. Hernberg. 1982. Mortality of 2,4-diclorophenoxy-acetic acid and 2,4,5-trichlorophenoxyacetic acid herbicide applicators in Finland. Scand. J. Work Environ. Health. 8:37-42. (Cited in Lynge, 1987)

Riihimaki, V., S. Asp, E. Pukkala et al. 1983. Mortality and cancer morbidity among chlorinated phenoxyacid applicators in Finland. Chemosphere. 12:565-571. (Cited in Lynge, 1987)

Rodwell D.E., R.D. Wilson, M.D. Nemec et al. 1989. Teratogenic assessment of 2,4-dichlorophenol in Fischer 344 rats. Fund. Appl. Toxicol. 13:635-640.

Saracci, R., M. Kogevinas, P-A. Bertazzi et al. 1991. Cancer mortality in workers exposed to chlorophenoxy herbicides and chlorophenols. The Lancet. 338(8774):1027-1032.

Storer, R.D., T.W. McKelvey, A.R. Kraynak et al. 1996. Revalidation of the *in vitro* alkaline elution/rat hepatocyte assay for DNA damage: Improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds. Mutat. Res. 368:59-101.

Terasaka, S., A. Inoue, M. Tanji and R. Kiyama. 2006. Expression of profiling of estrogen-response genes in breast cancer cells treated with alkylphenols, chlorinated phenols for evaluation of estrogenic activity. Toxicol. Letters. 163:130-141.

U.S. Air Force. 1983. An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Project Ranch Hand II. (Cited in Lynge, 1987)

U.S. EPA. 1980. Ambient Water Quality Criteria for 2,4-Dichlorophenol. Prepared by the Office of Water Regulations and Standards Criteria and Standards Division, Washington, DC, for the Office of Research and Development, Environmental Criteria and Assessment Office, Cincinnati, OH. EPA/440/5-80/042.

U.S. EPA. 1985. Drinking Water Criteria Document for Chlorinated Phenols. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Drinking Water, Washington, DC. (Cited in U.S. EPA, 2007)

U.S. EPA. 1987a. Health Effects Assessment for 2-Chlorophenol and 2,4-Dichlorophenol. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC.

U.S. EPA. 1987b. Health and Environmental Effects Document for Chlorinated Phenols. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

U.S. EPA. 1988. Recommendations for and Documentation of Biological Values for Use in Risk Assessment. Office of Health and Environmental Assessment, Cincinnati, OH. EPA/600/6-87/008. PB88-179874.

U.S. EPA. 1991. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. April.

U.S. EPA. 1994. Chemical Assessments and Related Activities. Office of Health and Environmental Assessment, Washington, DC. December.

U.S. EPA. 1997. Health Effects Assessment Summary Tables. FY-1997 Update. Prepared by the Office of Research and Development, National Center for Environmental Assessment, Cincinnati OH for the Office of Emergency and Remedial Response, Washington, DC. July. EPA/540/R-97/036. NTIS PB97-921199.

U.S. EPA. 2005. Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001F. Federal Register 70(66):17765--17817. Available online at http://www.epa.gov/raf

U.S. EPA. 2006. 2006 Edition of the Drinking Water Standards and Health Advisories. Office of Water, Washington, DC. Winter, 2004. EPA 822-R-06-013. Available at http://www.epa.gov/waterscience/criteria/drinking/dwstandards.pdf.

U.S. EPA. 2007. Integrated Risk Information System (IRIS). Online. Office of Research and Development, National Center for Environmental Assessment, Washington, DC. http://www.epa.gov/iris.

Vena, J., P. Boffetta, H. Becher et al. 1998. Exposure to dioxin and nonneoplastic mortality in the expanded IARC international cohort study of phenoxy herbicide and chlorophenol production workers and sprayers. Environ. Health Perspect. 106:645-563.

WHO (World Health Organization). 1989. Environmental Health Criteria 93: Chlorophenols Other than Pentachlorophenol. International Programme on Chemical Safety, Geneva, Switzerland. Available at http://www.inchem.org/documents/ehc/ehc/EHC093.HTM.

WHO (World Health Organization). 2003. Chlorophenols in Drinking-water: Background document for development of *WHO Guidelines for Drinking-water Quality*. WHO/SDE/WSH/03.04/47. Available at http://www.who.int/water_sanitation_health/dwq/chemicals/chlorophenols.pdf

Yamasaki, K., M. Takahashi and M. Yasuda. 2005. Two-generation reproductive toxicity studies in rats with extra parameters for detecting endocrine disrupting activity: Introductory overview of results for nine chemicals. J. Toxicol. Sci. 30:1-4.

Zeiger, E. 1990. Mutagenicity of 42 chemicals in *Salmonella*. Environ. Mol. Mut. 16(Suppl 18):32-54.

APPENDIX A. BENCHMARK DOSE MODELING OF CELL-MEDIATED IMMUNITY (MEAN FOOTPAD SWELLING) (EXON ET AL., 1984; EXON AND KOLLER, 1985)

The model fitting procedure for continuous data is as follows. The simplest model (linear) is first applied to the data while assuming constant variance. If the data are consistent with the assumption of constant variance ($p \geq 0.1$), then the fit of the linear model to the means is evaluated. If the linear model adequately fits the means ($p \geq 0.1$), then it is selected as the model for BMD derivation. If the linear model does not adequately fit the means, then the more complex models are fit to the data while assuming constant variance. Among the models providing adequate fit to the means ($p \geq 0.1$), the one with the lowest AIC for the fitted model is selected for BMD derivation. If the test for constant variance is negative, the linear model is run again while applying the power model integrated into the BMDS to account for nonhomogenous variance. If the nonhomogenous variance model provides an adequate fit ($p \geq 0.1$) to the variance data, then the fit of the linear model to the means is evaluated. If the linear model does not provide adequate fit to the means while the nonhomogenous variance model is applied, then the polynomial, power and Hill models are fit to the data and evaluated while the variance model is applied. Among those providing adequate fit to the means ($p \geq 0.1$), the one with the lowest AIC for the fitted model is selected for BMD derivation. If the test for constant variance is negative and the nonhomogenous variance model does not provide an adequate fit to the variance data, then the data set is considered unsuitable for modeling.

Following the above procedure, continuous-variable models in the EPA BMDS (version 1.3.2) were fit to the data shown in Table 3 (page 23) for decreased cell-mediated immunity (as measured by mean footpad swelling) in rats. Using these data, the constant variance model provided adequate fit to the variance data. With the homogeneous variance model applied, the linear model did not provide an adequate fit to the means, as shown in Table A-1. Further, none of the remaining models provided adequate fit to the data (there were not enough dose groups to apply the Hill model). In order to achieve model fit, the high dose group was dropped from the analysis. With the reduced data set, the homogenous variance model again fit the variance data adequately. With the homogenous variance model applied, the linear model provided adequate fit to the means (Figure A-1). The BMDs and the 95% lower confidence limits (BMDLs) associated with a change of 1 standard deviation (SD) from the control were calculated using the linear model with homogenous variance model applied.

Table A-1. Model Predictions for Footpad Swelling in Rats Exposed to 2,4-DCP (Exon et al. 1984; Exon and Koller, 1985)

Model	Variance *p*-value[a]	Means *p*-value[a]	BMD_{1sd} (mg/kg-day)	$BMDL_{1sd}$ (mg/kg-day)
All dose groups				
Linear (constant variance)	0.9413	0.04207	40.30	21.74
Polynomial (constant variance)[b]	0.9413	0.01183	40.30	21.74
Power (constant variance)[c]	0.9413	0.01183	40.30	21.74
Hill (constant variance)[c]	NA[d]			
Without high dose group				
Linear (constant variance)	0.8418	0.1747	3.21	1.84

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria
[b]Coefficients restricted to be negative; no adequate fit with any degree polynomial; 2-degree polynomial shown
[c]Power restricted to ≥ 1
[d]NA = not applicable (insufficient degrees of freedom available to fit this model)

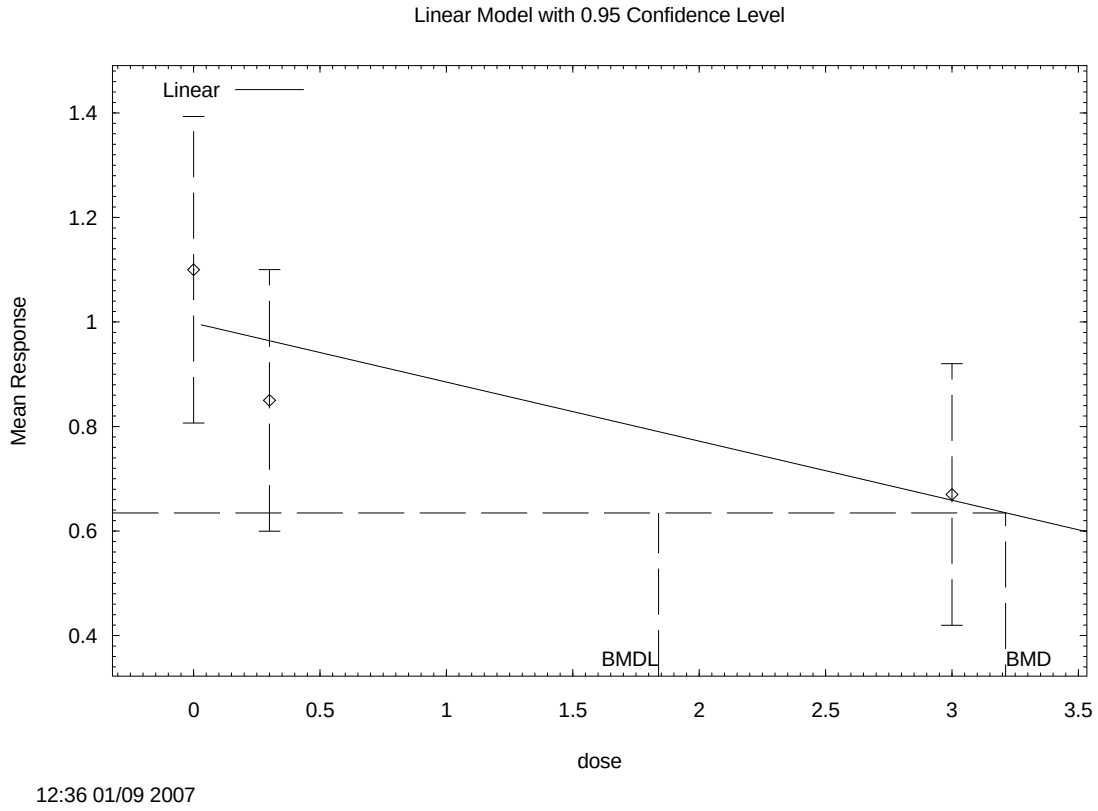

Figure A-1. Mean Footpad Swelling (mm) in Rats Exposed to 2,4-DCP (Reduced dataset) (Exon et al., 1984; Exon and Koller, 1985)

BMDs and BMDLs indicated are associated with a change of 1 SD from the control, and are in units of mg/kg-day.