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TOXICOLOGICAL REVIEW

OF

DICHLOROACETIC ACID

(CAS No. 79-43-6)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

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U.S. Environmental Protection Agency
Washington, DC

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to dichloroacetic acid (DCA). It is not intended to be a comprehensive treatise on the chemical or toxicological nature of DCA.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 202-566-1676.

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This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process

whereby the IRIS Program Director has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and the Regional Offices.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $µg/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for dichloroacetic acid (DCA) has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental*

Toxicity Risk Assessment (U.S. EPA, 1991a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), Draft Revised and Draft Final Guidelines for Carcinogen Assessment (U.S. EPA, 1999, 2003), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994b), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994c), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c) and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000d).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENE-TOX, DART/ETIC, EMIC, TOXLINE, CANCERLIT, and MEDLINE. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through January 2003.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Dichloroacetic acid (DCA) is a colorless to slightly yellow liquid, with a pungent acid-like odor. In aqueous solutions, DCA and its conjugate base, dichloroacetate, exist as an equilibrium mixture, the proportions of each depending primarily on the pH of the solution. With a pKa of 1.48 at 25°C, DCA occurs almost exclusively in the ionized form in normal drinking water (pH range 6-9). Other selected chemical and physical properties for this chemical are listed below (Merck Index, 1996; Lewis, 1997).

CASRN	79-43-6
Empirical Formula	$CHCl_2COOH$
Molecular Weight	128.94
Melting Point	13.5°C
Boiling Point	193-194°C
Density	1.5724 g/mL at 13°C
Physical State	Liquid
Solubility	Soluble in water, alcohol and ether
Specific Gravity	1.563 at 20/4 °C
Vapor Pressure	0.19 mbar (19 Pa) at 20°C

DCA has a very low vapor pressure and is not expected to volatilize from drinking water or contaminated environmental media to any appreciable extent. Therefore, inhalation exposure from volatilized DCA is negligible and is not considered in this document.

3. TOXICOKINETICS

3.1. ABSORPTION

Studies in humans and animals indicate that DCA is readily absorbed by the gastrointestinal tract (Lin et al., 1993; Larson and Bull, 1992; Stacpoole et al., 1998a). Following oral administration of radiolabeled DCA to rats and mice, only about 1-2% of the label was found in the feces, indicating almost complete gastrointestinal absorption (Lin et al., 1993; Larson and Bull, 1992). In fasted human subjects, peak plasma DCA concentration occurs within 15 to 30 minutes of oral dosing (Stacpoole et al., 1998a).

3.2. DISTRIBUTION

Lin et al. (1993) and James et al. (1998) investigated the distribution of absorbed DCA to internal tissues in rats. Lin et al. (1993) reported that 48 hours after oral DCA administration (gavage), 21 to 36% of the tracer [^{14}C] was recovered from tissues, with the precise amount dependant on the dose as well as the form of labeled DCA that was administered. The majority of tracer was found in the liver, muscle, skin, blood and intestines. At 24 hours, all of the other tissues combined (kidney, adipose, stomach, testis, lung, spleen, heart, brain, and bladder) contained 10 to 15% of the label (James et al., 1998) while at 48 hours these tissues contained ~1-2% of the original dose given (Lin et al., 1993).

3.3. METABOLISM

The primary metabolic pathway for DCA involves oxidative dechlorination to form glyoxylate (Larson and Bull, 1992). This reaction, once thought to be microsomal Cytochrome P-450 mediated, has now been shown to be NADPH- and GSH-dependent and occurs predominantly in the cytosol (Lipscomb et al., 1995; Cornett et al., 1997; Stacpoole et al., 1998a; Board et al., 1997). Recent work by Tong et al. (1998 a; 1998 b) has identified a rat liver cytosolic enzyme, glutathione-S-transferase Zeta (GST Zeta), that catalyzes the conversion of DCA to glyoxylate. This enzyme is considered the rat ortholog of human GST zeta.

Data on DCA metabolism in humans are available because DCA has been used experimentally in the therapeutic treatment of several metabolic disorders. The data obtained support the hypothesis that DCA metabolism is similar in both humans and rodents (Stacpoole et

al., 1998a). The occurrence of oxalic acid in the urine of DCA-treated patients indicates that DCA is oxidatively dechlorinated to glyoxylate, which is then converted to oxalate. In one child with congenital lactic acidosis, monochloroacetic acid was present in plasma in addition to oxalate and glyoxylate during the first four hours after the initial dose. Monochloroacetic acid concentrations were then below detection for the remainder of the observation period. Initially, the concentration of monochloroacetic acid in plasma exceeded that for glyoxylate, but not oxalate (Stacpoole et al., 1998a). These data indicate that in at least some individuals, the reductive dechlorination pathway can occur initially after DCA administration, but continued DCA metabolism occurs through the oxidative dechlorination pathway.

GSTZ appears to be identical to maleylacetoacetate isomerase (MAAI), the enzyme in the pathway for tyrosine catabolism that converts the *cis* double bond in maleylacetoacetate (MAA) to the *trans* double bond in fumarylacetoacetate, using GSH as a cofactor (Fernandez-Canon and Penalva, 1998). GSTZ/MAAI appears to have an active site geometry that is highly-conserved across species and is sufficiently-plastic that it can participate in *cis/trans* isomerization reactions and dehalogenation of molecules as diverse as DCA and pentachlorophenol. It also has moderate GSH peroxidase activity (Anandarajah et al., 2000; Polekhina et al., 2001; Sheehan et al., 2001). In humans GSTZ is expressed mostly in the liver followed by kidney, and skeletal muscle. It is also expressed in the placenta, heart, pancreas, mammary tissues, seminal glands, and fetal liver (Fernandez-Canon et al., 1999; Polekhina et al., 2000). The complete pathway for tyrosine catabolism is found only in the liver and kidney. The presence of the enzyme in other tissues suggests that it has functions other than the isomerization of MAA (Fernandez-Canon et al., 1999).

There are species and age-related differences in the activity of GSTZ. The relative rate of DCA transformation in mouse hepatic cytosol was greater than in rat hepatic cytosol which in turn was greater than in human hepatic cytosol (Tong et al., 1998a). The K_m and V_{max}/K_m values for DCA in mice were 81.9 ± 5.6 μM and 52.9 ± 2.46 ($x10^{-3}$) respectively, those in rats were 70.1 ± 5.3 μM and 32.4 ± 4.87 ($x10^{-3}$), and those in humans were 47.3 ± 6.7 μM and 8.25 ± 1.37 ($x10^{-3}$) (Tong et al., 1998a). K_m and V_{max}/K_m values for DCA transformation in naive young mice were 108 μM ± 16 and 6.72 ml/hr/mg, while those for aged mice were 56.1 μM ± 14.2 and 8.92 ml/hr/mg, demonstrating a difference in the response of the enzyme in the young versus the older mice (Schultz et al., 2002).

Among humans there are known polymorphisms in GSTZ which may account for differences in the ability to metabolize DCA and other halogenated compounds (Sheehan et al., 2001). The polymorphisms result from A/G transitions at nucleotides 94 and 124 of the coding region and T/C transitions at positions 23 and 245 (Blackburn et al., 2000, 2001). The GSTZ variants are the products of the different combinations of the bases at the variant positions and were designated GSTZ1a-1a, GSTZ1b-1b, GSTZ1c-1c, GSTZ1d-1d, and GSTZ1e-1e (Blackburn et al., 2000, 2001; Tzeng et al., 2000). Analysis of a Caucasian (unselected, European Australian blood donors) population (141 subjects: 68 females and 73 males, ages 16 to 69) by Blackburn et al. (2000) showed that the first three allele variants were present with frequencies of 0.09, 0.28, and 0.63, respectively. Blackburn et al. (2001) reported the results of an analysis for five variants in a similar population of 128 subjects where the variant distribution was 0.086, 0.285, 0.473, 0.156 and 0 for GSTZ1a-1a, GSTZ1b-1b, GSTZ1c-1c, GSTZ1d-1d, and GSTZ1e-1e, respectively. GSTZ1a-1a has been demonstrated to have different catalytic properties toward DCA than the other variants, including a 4-5-fold higher activity. However, excluding the GSTZ 1e-1e variant, the most active human GSTZ variants toward the catabolism of DCA appeared at the lowest frequency in the populations studied by Blackburn et al. (2000, 2001). The most common variant, GSTZ1c-1c, had the highest activity toward the isomerization of MAA (MAA is chemically too unstable to be used in the enzyme studies) using maleylacetone as a surrogate.

Glyoxylate formed from the metabolism of DCA may be routed though several different pathways (Figure 3-1). Transamination by peroxisomal alanine-glyoxylate transaminase forms glycine, which can be incorporated into proteins, used in the synthesis of serine, or degraded releasing carbon dioxide. Conversion to oxalate occurs via a (S)-2-hydroxyacid dehydrogenase such as lactate dehydrogenase. Glyoxylate can also be converted to glycolate by glyoxylate reductase (Michal, 1999).

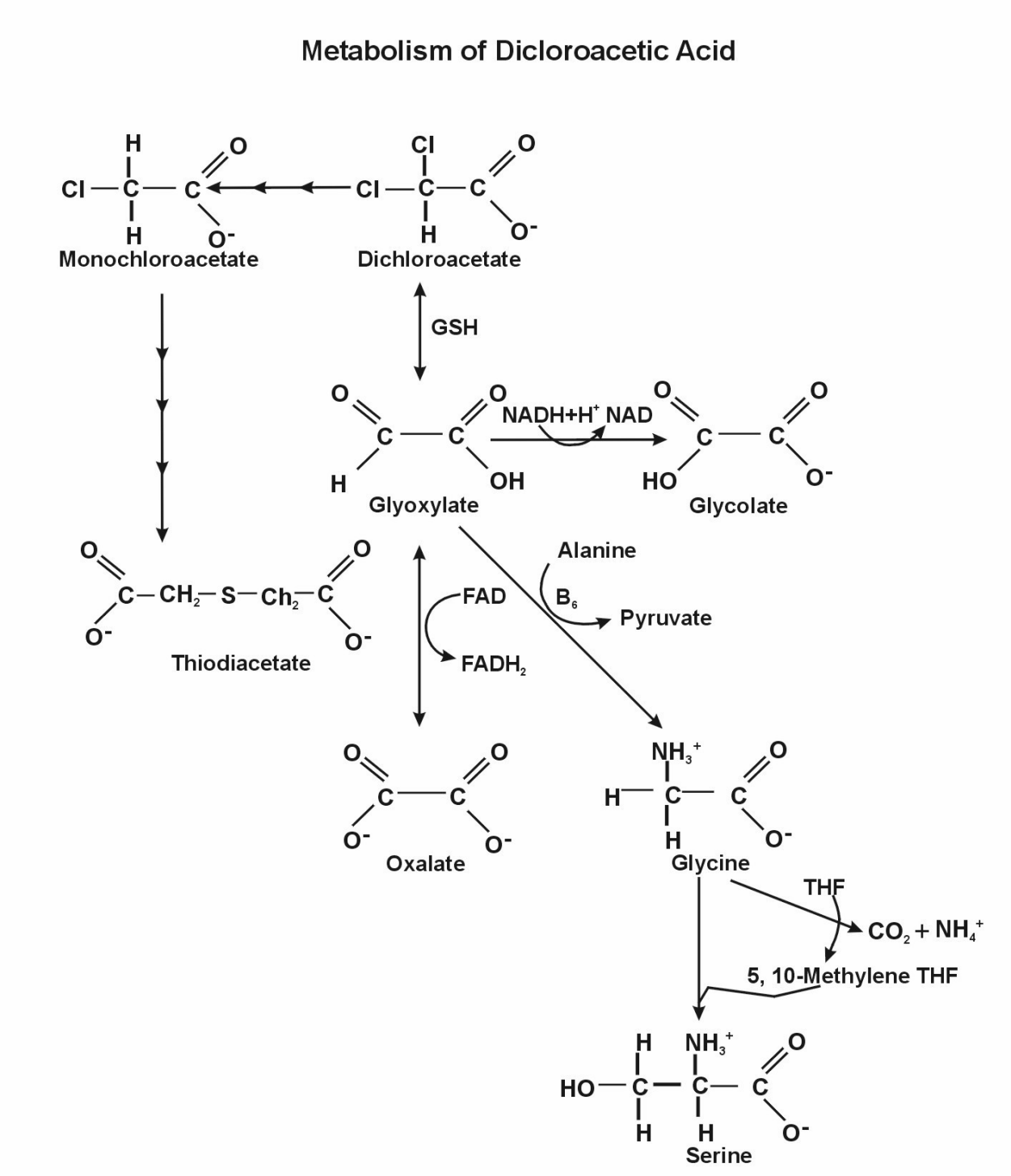

Figure 3-1. Metabolism of DCA

Source: Adapted from Michal, 1999.

There may be other metabolic pathways for DCA. Oxalate, glycine, CO_2, glycolate, monochloroacetic acid and thiodiacetic acid have been shown to be metabolites of DCA in rodents, although the relative amount of each seems to be species-specific (Larson and Bull, 1992; Lin et al., 1993; Gonzalez-Leon and Bull, 1996; Xu et al., 1995). While urinary metabolites (glyoxylate, glycolate, oxalate, monochloracetic acid, and thiodiacetic acid) account for ~12-30% of the administered dose in rats and mice, CO_2 excretion may differ between these two species. Larson and Bull (1992) report that exhaled CO_2 generated from radiolabeled DCA was approximately 24 to 30% of a single administered dose in rats, but represented only 2% of the same dose in mice. However, a later study (Xu et al., 1995) indicates that approximately 45% of a single administered dose of DCA in mice was exhaled as CO_2 in the first 24 hours after dosing. A problem associated with the recovery of label in the Larson and Bull (1992) study may have resulted in the lower value in mice. In both species the nonchlorinated acids were the primary metabolites detected in urine. Thiodiacetic acid concentrations were much greater than monochloroacetic acid, which was present in only trace quantities (Larson and Bull, 1992).

To account for the production of metabolites that are not metabolically linked to glyoxylate, Stacpoole et al. (1998a) and Larson and Bull (1992) proposed reductive dechlorination of DCA yielding monochloroacetic acid as an alternate metabolic pathway. The monochloroacetic acid is converted to thiodiacetic acid via glutathione conjugation. While it has been speculated that this pathway might involve the formation of free radicals (Larson and Bull, 1992), it has not been investigated (Stacpoole et al., 1998a).

DCA metabolites (e.g., glyoxylate) can enter intermediary metabolism, and the carbon atoms originally present in DCA can become incorporated into endogenous proteins and other biomolecules. Stevens et al. (1992) investigated this possibility and reported that a substantial portion of a radiolabeled dose of DCA was not excreted, but was oxidatively metabolized into glycine and incorporated into serum albumin. These data are consistent with the results from a study by Larson and Bull (1992), who reported that within 3 hours of dosing mice and rats, high concentrations of radiolabel were incorporated into various plasma proteins.

One of the unique features of DCA toxicokinetics is the ability of the compound to inhibit its own metabolism. Lin et al. (1993) administered single oral doses of 100 and 282 mg/kg to rats, and measured urinary output of the parent compound. At the low-dose, only 1-2% of the administered DCA appeared in the urine. However, with the 282 mg/kg dose, 20% of the parent compound was excreted, suggesting that the metabolic capacity of rats was exceeded in

the high-dose range. Similar effects have been reported in healthy human volunteers treated with DCA, indicating that inhibition also occurs in humans (Stacpoole et al., 1998a).

Plasma clearance rates for DCA can vary substantially among species. Following a single oral dose of approximately 50 mg/kg in humans, the plasma half-life of DCA was 0.5-2 hours, with less than 1% of the parent compound excreted in the urine (Lukas et al., 1980; Curry et al., 1991). This was similar to results reported in mice and rats (Lin et al., 1993; Larson and Bull, 1992; James et al., 1998). Although Lukas et al. (1980) found that rats cleared DCA from their blood with a half-life of 2.0-4.4 hours following intravenous injection of 100 mg/kg, clearance of the radiolabel from the ingested DCA metabolites took a much longer time (21-36 hours). Dogs clear DCA from their blood at a slower rate. Following injection of 100 mg/kg DCA, Lukas et al. (1980) found that initial blood levels of DCA in two dogs were approximately double those in three treated rats. DCA concentrations fell from their peak levels to half the concentration at some point between 6 and 24 hours leading to an estimated half-life of 17.1 to 24.6 hours. Sampling from one dog was discontinued at 24 hours because of collapsing veins. However, at 48 hours, the rats and the remaining dog had approximately the same percent of the initial DCA concentration as residual in plasma (10-20% for the three rats and 10% for the dog).

Prior exposure to DCA significantly inhibits its metabolism (Curry et al., 1991; Gonzalez-Leon et al., 1997a, b, 1999; Lukas et al., 1980; Schultz et al., 2002). Studying the plasma half-life of DCA in human volunteers, Curry et al. (1985) found that the mean half-life of DCA increased from 63.3 minutes to an average of 374 minutes following the fifth in a series of 50 mg/kg doses administered intravenously at 2-hour intervals. In another study (Stacpoole et al., 1998a) of healthy adults in which 25 mg/kg DCA was administered daily for five days, the half-life increased about eightfold on the fifth day when compared to the first (1.09 ± 0.45 hr vs. 8.03 ± 5.62 hr). The most likely basis for the decrease in DCA clearance observed with repeated- or high-dose exposure is the inactivation of one or more of the enzymes involved in its metabolism.

Recent work by Tong et al. (1998a) demonstrates that prior DCA exposure in rats substantially reduces the cytosolic conversion of DCA to glyoxylate from the inhibition of GSTZ. The rate constants for the DCA-dependent inactivation of the four polymorphic variants of recombinant human GSTZ were in the following order: variant 1a-1a < 1b-1b ≈1c-1c ≈1d-1d (Tzeng et al., 2000). Thus, the most frequent human GST variant (GSTZ1c-1c) observed by Blackburn et al. (2000) has a low activity toward DCA and is impacted by DCA inhibition to a

greater extent than the most active enzyme variant (GSTZ1a-1a). The observations of Tzeng et al. (2000) were confirmed by Lantum et al. (2002) where each of the four enzyme variants were tested *in vitro* with chlorofluoroacetate as the substrate. Residual enzyme activities of the 1b-1b, 1c-1c, and 1d-1d variants were 3, 4.5, and 4% of the original activities while 1a-1a retained 12% of its original activity. Accordingly, one might expect poor clearance of DCA from human plasma via oxidative dechlorination when exposure is continuous even in individuals that carry the 1a-1a GSTZ variant.

Work by Anderson et al. (1999), Tong et al. (1998a), and Wempe (1999) suggests that the inhibition of GSTZ is due to the formation of a covalent bond between GSH and DCA forming an S-(α-chlorocarboxymethyl) glutathione intermediate. This intermediate can then undergo hydrolysis releasing the remaining chloride and forming S-(α-hydroxycarboxymethyl) glutathione liberates glyoxylate and regenerates the GSH. Alternately, S-(α-hydroxycarboxymethyl) glutathione reacts with a nucleophilic residue on the enzyme (i.e., histidine or tyrosine) and modifies and inhibits the enzyme. Anderson et al. (1999) demonstrated that the inactivation of GSTZ by DCA is irreversible and is accompanied by a loss of immunoreactive GSTZ protein. Additional support for irreversible enzyme inhibition is provided by the work of Schultz et al. (2002), which demonstrates that the recovery from enzyme inhibition requires protein synthesis.

Schultz et al. (2002) found that the metabolic clearance of DCA in mice depends on age, dose, and the presence or absence of pretreatment. For example, pretreatment of the animals with increasing DCA doses decreased metabolic clearance based on *in vitro* kinetic measurements of hepatic enzymes. However, the metabolism of DCA by pre-exposed older rats was comparable to the older naive rats. In hepatic tissues of young mice, decreased metabolic clearance was accompanied by a decrease in immunoreactive GST zeta, while in aged mice the amount of immunoreactive protein remained constant (Schultz et al., 2002). The authors hypothesized that this was the result of decreased turnover of the inhibited enzyme with increasing age. Aged rats (16-month) showed a decreased capacity to metabolize the second of two doses of DCA when compared to rats that were three to four months old (James et al., 1998). The aged rats also had peak plasma concentrations that were 5-fold higher than the young rats, while elimination half-life was approximately doubled.

Additional evidence to support enzyme inhibition comes from studies in which rodents were predosed with DCA in their drinking water for 2 weeks, followed by a single intravenous

dose of DCA. This predosing regimen significantly lowered DCA-derived CO_2 excretion in rats, but not in mice (Cornett et al., 1997). The predosing regimen also resulted in a significant increase in plasma area under the curve (AUC) values for the parent compound relative to controls in rats and mice (Cornett et al., 1997). A similar study by Gonzalez-Leon et al. (1999) found no significant change in the amount of labeled CO_2 formed in mice treated with 2 g/L DCA in drinking water for two weeks and a subsequent 100 mg/kg dose of DCA by gavage. The plasma AUC for DCA was about three times that for the untreated controls. These results in mice support the findings of Cornett et al. (1997).

Schultz et al. (2002) conducted an experiment in young (8-week-old) mice by exposing them to 2.0 g/L DCA for 14 days and an intravenous dose of 20 mg/kg DCA at 6-, 16-, 36-, or 48-hours after cessation of the drinking water exposure. Metabolic clearance of DCA was greatly inhibited 6 and 16 hours after the end of the drinking water exposure periods, slightly inhibited 36 hours after the end of the drinking water exposure and essentially the same as untreated controls 48 hours after the end of the DCA drinking water exposure period. A similar inhibition of metabolism of subsequent DCA doses after the administration of a 50 mg/kg dose has been shown in rats (James et al., 1998).

The toxicological relevance of the inhibition of glutathione-S-transferase biotransformation by DCA in different species is not entirely clear. For instance, DeAngelo et al. (1996) determined that Fischer (F344) rats were more sensitive than B6C3F1 mice with regard to DCA-induced hepatocarcinogenicity based on the mean daily doses at which 50% of the animals exhibited liver neoplasia (Tong et al., 1998a). However, the rates of DCA biotransformation were much greater in mice than rats. Accordingly, Tong et al. (1998a) concluded that the carcinogenicity of DCA does not appear to be directly related to its glutathione-S-transferase-dependent biotransformation.

Cornett et al. (1999) suggested that differences in carcinogenicity may be related to tyrosine metabolites that accumulate when GSTZ is inhibited rather than DCA metabolites. The study authors proposed that DCA concentrations that inhibit GSTZ also increase the concentration of MAA and its decarboxylated end product, maleylacetone, both of which are postulated to be alkylating agents and are linked to the mechanism for carcinogenesis for those that suffer from hereditary tyrosinemia I (Schultz et al., 2002).

3.3.1. Mechanistic Metabolic Considerations

There are a number of unanswered questions about the metabolism of DCA and its relevance to toxicity in laboratory species and humans. The question of whether or not there is more than one metabolic pathway for DCA remains unanswered. Recent work by Schultz et al. (2002) comparing DCA clearance in pre-exposed young (10-week-old) versus aged (60-week-old) mice suggests that there may be more than one metabolic option in mature animals. In the aged mice, DCA clearance was minimally affected by pretreatment with DCA, but it was significantly suppressed (>80%) in the young mice with recovery times of less than 16 hours. This is in contrast to the *in vitro* work that had demonstrated that the hepatic GSTZ was inhibited in the aged mice to almost the same extent as in the young mice and suggested the possibility of extrahepatic metabolism in the older mice.

The relative affinities of the GSTZ/MAAI active site for DCA versus maleylacetoacetate, when DCA concentrations are low, is another consideration that needs investigation. Maleylacetoacetate is a degradate of phenylalanine and tyrosine, both essential amino acids in mammals. Because of this, the activity of GSTZ with MAA as a substrate may be favored over that of GSTZ with DCA at low concentrations such as those present in chlorinated water. This could favor DCA metabolism by another pathway. Lantum et al. (2002) examined GSTZ activity using compounds similar to MAA and DCA that provide information on the relative variant activities. Maleylacetone was used as a surrogate for maleylacetoacetate while chlorofluoroacetate was considered as a surrogate for DCA.

The studies by Lantum et al. (2002) were conducted *in vitro* with the 1a-1a, 1b-1b, 1c-1c, and 1d-1d enzyme variants. Reactions with the substrates (maleylacetone -- 0 to1 mM or chlorofluoroacetate -- 0 to 2 mM) were carried out at pH 7.4 and 25°C for 30 seconds with measurement of product by HPLC. Triplicate samples were analyzed and the values for K_m, k_{cat}, V_{max} and k_{cat}/K_m were determined. There was considerable variability in the V_{max} and K_m values with maleylacetone as the substrate as reflected in the standard estimates of the means. Enzyme activity seemed to be driven by the k_{cat} differences to a greater extent than the K_m values. In other words, the catalytic activity in the active site (k_{cat}) appeared to impact V_{max} to a greater extent than the affinity of the active site for the substrate (K_m).

Despite the variability of results, the lower catalytic efficiency of the 1a-1a variant with maleylacetone as a substrate was apparent in the k_{cat} values as was its greater efficiency with chlorofluoroacetate. The 1c-1c variant had the highest k_{cat} with maleylacetone as a substrate. The k_{cat}/K_m ratios (Table 3-1) also reflect the lower effectiveness of 1a-1a with maleylacetone as

a substrate but the ratios are fairly consistent for the chlorofluoroacetate, suggesting comparable effectiveness of the enzyme variants when substrate concentrations are below saturation.

Table 3-1. Enzyme kinetics for GSTZ: k_{cat}/K_m ratios ($M^{-1}sec^{-1}$)

Enzyme Variant	Substrate	
	maleylacetone	chlorofluoroacetate
GSTZ 1a-1a	7.6×10^5	4.3×10^3
GSTZ 1b-1b	20×10^5	4.7×10^3
GSTZ 1c-1c	14.5×10^5	5.0×10^3
GSTZ 1d-1d	20.6×10^5	4.1×10^3

Source: Adapted from Lantum et al. (2002).

Lantum et al. (2002) also evaluated the inhibitory effect of maleylacetone and its product, fumarylacetone, on the reaction of chlorofluoroacetate. Lineweaver-Burke plots of the inhibition indicate that it was neither purely competitive nor noncompetitive. Accordingly, the authors described the effects of maleylacetone and fumarylacetone as mixed inhibition.

The work of Lantum et al. (2002) suggests that GSTZ would preferentially react with MAA under conditions where DCA and MAA were competing for the enzyme's active site in individuals possessing the 1b-1b, 1c-1c, and 1d-1d variants and that there would be a greater opportunity for haloacetic acid to be favored with the 1a-1a variant. However, it is important to recognize that the reactions were carried out under conditions where there had been no preexposure of the enzymes to a halo-acid and, thus, no prior inhibition. Results with an inhibited enzyme might be quite different.

The identity of the DCA toxic intermediate(s) for cancer and noncancer effects is also unknown. As mentioned previously, Cornett et al. (1999) suggested that MAA and maleylacetone, the tyrosine metabolites that could accumulate when GSTZ is inhibited, might be involved with DCA toxicity because they are alkylating agents. Fernandez-Canon et al. (2002) demonstrated that there is a metabolic bypass to this reaction in MAAI/GSTZ-deficient mice.

Homozygous-MAAI null mice were monitored for up to 22 months and displayed normal growth and reproductive success when compared to the controls. No adverse effects on tissue histopathology were seen at two and six months in the organs examined (including liver and

testes). Some biochemical abnormalities were observed. For example, the authors determined that fumarylacetoacetate and succinylacetone were found in the urine of the enzyme-deficient mice, but not in the controls and that there was an induction of NMO-1 mRNA in the liver in the MAAI deficient mice. NMO-1 has the ability to reduce quinones via a mechanism that prevents the generation of free radical oxygen thereby protecting cells from oxidative stress.

Another important difference between the enzyme-deficient mice and the controls was their response to a diet enriched in phenylananine, tyrosine ,or protein. Increased intake of protein, phenylalanine, or tyrosine caused a rapid loss of weight and death in the enzyme-deficient mice. This study serves to reduce but not remove the concern that the toxicologically-active metabolite in DCA-exposed mice is MAAI or maleylacetone, rather than DCA or a DCA metabolite. The presence of the bypass reaction, however, may not be completely protective for species such as humans that consume a high-protein diet.

Carcinogenic and genotoxic effects of DCA have been most strongly associated with high doses where DCA metabolism is inhibited. This observation may indicate that DCA or a metabolite produced when the availability of the GSTZ pathway becomes limiting is the most actively toxic compound. Tzeng et al. (2000) reported that the relative rate of DCA-induced inactivation of liver GSTZ was greater in rats than in mice or humans. GSTZ activity was greater in mouse liver than human liver. This could mean that humans are more sensitive to DCA toxicity than other species if toxicity is due to unmetabolized DCA.

Dose is another factor to consider in evaluating the toxicity of DCA in acutely- and chronically-exposed subjects. Saghir and Schultz (2002) examined the oral bioavailability of DCA in rats at doses of 0.25 to 100 mg/kg. Previously unexposed animals were given 1, 5 or 20 mg/kg DCA; blood samples were collected and analyzed for DCA at intervals over a 24-hour postdosing period. DCA was rapidly metabolized for the 1 mg/kg dose and plasma concentrations were less than 6 ng/mL (the limit of detection) within 15 minutes of dosing. With the 5, 20, and 100 mg/kg doses, the amounts of DCA in the plasma (oral bioavailability) were 10, 13 and 81% of the dose, respectively. In rats that had been pretreated with DCA in drinking water (0.2 mg/L) for seven days to inhibit GSTZ, the estimates of the oral bioavailability were 14, 28, 31, 75 and 100%, respectively, for oral doses of 0.25, 1, 5, 20, and 100 mg/kg.

3.4. ELIMINATION

Only a small fraction of DCA (~1-2%) is found in the feces in animal studies (Lin et al., 1993; Larson and Bull, 1992). There is also minimal (~1%) excretion of unmetabolized DCA in the urine at low doses (Lukas et al., 1980; Curry et al., 1991; Lin et al., 1993), but as the DCA dose increases the amount of parent compound in the urine also increases (Lin et al., 1993).

Kim et al. (1999) collected morning urine samples from 25 women who were part of a study on neural tube defects and whose drinking water was chlorinated. Exposure to DCA was estimated based on analysis of a single tap water sample from the subjects home. Subjects spent most of their time in their household for the 48-hour study period. While DCA was detected in the urine of all subjects, there was no relationship between estimated exposure and urinary DCA excretion or creatinine-normalized DCA excretion. As part of the same study, two women ingested water containing 4.0 or 6.3 μg/L DCA. DCA appeared in the urine immediately after exposure and accounted for 2 to 5% of the ingested dose. This amount is a slightly higher fraction of the ingested dose than has been reported for animals.

Oxalate is the primary urinary metabolite of DCA; it is formed by the oxidation of glyoxylate (Stacpoole et al., 1998a). In humans and animals, variable quantities of glyoxylate, glycolate, monochloracetic acid, and thiodiacetic acid are found in the urine (Larson and Bull, 1992; Lin et al., 1993; Gonzalez-Leon and Bull, 1996; Stacpoole et al., 1998a). A fraction of the glyoxylate produced from DCA is oxidized to carbon dioxide and is exhaled. Carbon dioxide is also produced by the degradation of glycine formed from glycoxylate.

3.5. PHYSIOLOGICALLY-BASED TOXICOKINETIC MODELS

A pharmacokinetic model for DCA used Advanced Continuous Simulation Language and data from B6C3F1 male mice exposed to DCA by intravenous injection and oral gavage (Barton et al., 1999). Some of the tested animals had no prior exposure to DCA, while others had been pretreated with drinking water containing 2 g/L for two weeks prior to the administration of 20 or 100 mg/kg test doses. A two-compartment model was developed to project expected blood concentrations and area under the curve in the liver (AUCL) after DCA exposure; the model included compartments for the lumen of the small intestine, the liver, and the body (with its volume of distribution corrected for the liver volume). DCA uptake by way of intravenous, injection, gavage, and drinking water exposures plus elimination rates via hepatic metabolism and excretion were included in the model. Experimental data were used to determine the volume

of distribution, metabolic rate, uptake kinetics, and elimination constant that would fit the data in the model.

The control animals were found to clear DCA from blood rapidly (metabolic rate: 40 mg/hr/kg body weight). The rate of clearance for the preexposed mice was considerably lower (metabolic rate: 3-8 mg/hr/kg body weight) resulting in higher blood concentrations of DCA in these animals (Barton et al., 1999). While the model seemed to fit the data, it under-predicted blood concentrations for mice intravenously exposed to 100 mg/kg at about 1 hour post exposure for naive and pretreated mice. The projected clearance time from blood for the naive mice was 1 hour while it was 3.5 hours in the pretreated mice, reflecting the inhibition of DCA metabolism.

For mice exposed via drinking water, the model predicted a nonlinear AUCL for both naive and pretreated animals (Barton et al., 1999). This reflects saturation of metabolism in both instances. The projected AUCL was higher in the pretreated animals than in the naive animals with drinking water concentrations of 0.01 to about 100 g/L. The model projected an AUCL for the pretreated animals that was about 8-fold higher than the untreated animals at concentrations between 0.01 and about 0.8 g/L. With drinking water concentrations between 1 and 10 g/L, the modeled difference between the naive animals and the pretreated animals increased dramatically to a greater than 200-fold difference and then narrowed until it was the same for both groups at concentrations of about 100 g/L.

The objective for the development of the DCA pharmacokinetic model was to provide a mechanism for estimating liver concentrations of DCA that would be useful in refining the tissue dose-response for liver tumors. The model has some utility in projecting liver concentrations under conditions where the metabolism of DCA is not inhibited and again under conditions of maximum inhibition. However, it cannot provide estimates under conditions of partial metabolic inhibition or project how liver concentrations might vary with differences in the activity of GSTZ isozymes.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS

The following studies in humans are comprised of clinical reports and case studies. There are currently no epidemiological studies regarding the chronic effects of DCA exposure in humans with numbers adequate to provide information regarding carcinogenicity or toxicity at doses lower than those discussed in the following sections. Studies in humans cannot predict carcinogenicity in the exposed individuals because of several limitations: in most studies too few individuals were studied, the exposure period or observation period was limited and a minimum number of endpoints were monitored. The reader is therefore advised to consider the following information as indicative only of potential toxicity in humans exposed to DCA at therapeutic levels. Current studies are inadequate to support predictions regarding potential adverse effects in humans exposed to DCA at concentrations approximating those currently detectable in finished drinking water.

For over 25 years, DCA has been used clinically as an investigational drug to treat several metabolic disorders (congenital lactic acidosis, familial hypercholesterolemia, and diabetes). At the present time the most active pharmaceutical use of DCA is its application in the treatment of congenital lactic acidosis; applications in the treatment of diabetes and hypercholesteremia do not appear to have continued. Congenital lactic acidosis includes a group of inborn metabolic disorders that result in increased blood lactate concentrations. In most cases the metabolic defect is located in the pyruvate dehydrogenase complex, but it can also involve enzymes in the citric acid cycle, enzymes in the respiratory chain, pyruvate carboxylase or phosphoenolpyruvate kinase (Stacpoole et al., 1998b). Each of these enzymes is involved either in bridging the end products of glycolysis to the citric acid cycle or in mitochondrial oxidative metabolism. Affected children exhibit accumulation of lactate and hydrogen ions in blood, urine or cerebrospinal fluid, failure to thrive, and neuromuscular degeneration. Approximately 250 new cases are identified per year and there is about a 20% annual mortality rate for the affected population (Stacpoole et al., 1998b). Some cases of congenital lactic acidosis do not respond to DCA treatment.

Effects of DCA treatment have been limited to transient central neuropathy (sedation), peripheral neuropathy (tingling in fingers and toes and nerve conduction changes), and metabolic

changes such as decreases in fasting glucose, plasma lactate and cholesterol, and alanine. For example, Stacpoole et al. (1978) studied diabetic or hyperlipoproteinemic patients, ranging in age from 42 to 71 years. They each received a daily oral dose of 3 to 4 g DCA (43 to 57 mg/kg-day, assuming a 70-kg body weight) for 6 or 7 days. Seven female patients were studied over the subsequent 7 days, while four patients (three female, one male) were studied in more detail over a 15-day period after treatment. Some patients experienced mild sedation, but no other laboratory or clinical evidence of adverse effects were noted either during or immediately after the treatment phase.

Dichloroacetate treatment significantly reduced fasting blood glucose levels an average of 24% and produced marked, concomitant decreases in plasma lactate (73%) and alanine (82%) (Stacpoole et al., 1978). Plasma cholesterol levels significantly decreased (22%) and triglyceride levels decreased by 71%. Plasma insulin, free fatty acid, and glycerol levels were not altered. The treatment also depressed uric acid excretion, resulting in elevated serum uric acid levels. Maximum effects were generally noted at the end of the 6- to 7-day treatment period and returned to pretreatment levels during the post-treatment observation period. Plasma cholesterol levels were not altered by treatment in one patient, and the depression of cholesterol levels in the others returned to the pretreatment levels during the recovery period.

The effects of DCA on intermediary metabolites appear to be the result of its activation of pyruvate dehydrogenase, a key enzyme controlling the flow of three carbon metabolites into the citric acid cycle. Pyruvate dehydrogenase exists in active and inactive forms, and is deactivated by phosphorylation through the action of pyruvate dehydrogenase kinase. It is activated through the removal of the phosphate via pyruvate dehydrogenase phosphatase. DCA is an inhibitor of the kinase, thus maintaining the enzyme in its active form (Stacpoole et al., 1998a).

Moore et al. (1979) evaluated clinical effects in two individuals treated with dichloroacetate for radically elevated serum cholesterol. An 8-year-old boy with severe familial hypercholesteremia was given 50 mg/kg-day DCA orally. Total serum cholesterol levels decreased from >1,000 to 849 mg/dL within 7 days. Continued treatment for 5 weeks resulted in a further decrease to 727 mg/dL. No adverse clinical or laboratory signs were detected in this individual.

In a case study of a 21-year-old man reported by Moore et al. (1979), dichloroacetate treatment (50 mg/kg-day) decreased total serum cholesterol levels from 578 to 372 mg/dL in 1 week. At this point, the patient was switched to therapy with nicotinic acid and cholestyramine, but treatment was ineffective and cholesterol levels rose to more than 500 mg/dL. Therapy was reinstated and serum cholesterol levels decreased to 363 mg/dL after 2 weeks and to 325 mg/dL after 10 weeks. After 16 weeks of treatment, the patient complained of tingling in his fingers and toes. Physical examination revealed slight decreases in the strength of facial and finger muscles, diminished to absent deep tendon reflexes, and decreased strength in all muscle groups of the lower extremities (distal muscle groups being most severely affected). Electromyographic studies revealed denervation changes in foot and distal leg muscles. Mild slowing of conduction velocity was noted in both posterior tibial nerves, and no measurable response was obtained in the peroneal or sural nerves. Treatment was immediately discontinued. Eight weeks after treatment stopped, the patient stated that the tingling sensation had subsided. The strength of his facial muscles was normal, and strength in his legs and feet was slightly improved. Six months after treatment was stopped, the patient exhibited normal motor strength, increased deep tendon reflexes and marked improvement in electromyographic and nerve conduction examinations. Serum cholesterol returned to its former high level following the cessation of treatment.

Stacpoole et al. (1998a, b) reviewed observations in humans that have accrued from nearly 25 years of experimental DCA clinical use, primarily in the treatment of congenital lactic acidosis. Therapeutic doses of DCA are usually in the range of 25-50 mg/kg-day (either oral or intravenous). In several cases, treatments at 25 mg/kg-day have occurred for as long as 5 years. Evidence of clinically-significant DCA toxicity in humans is primarily limited to the central and peripheral nervous system. Approximately 50% of patients receiving 25-50 mg/kg-day experience sedative effects. This effect is observed following oral, intravenous, or repeated dosing regimens. There have been three reported cases of peripheral neuropathy following DCA treatment, but all were completely reversible within 6 months of cessation of treatment. In one case, following the reversal of neurological symptoms, reinstitution of DCA at 10 to 25 mg/kg-day was maintained for 2 years without further evidence of neuropathy. Two children that were treated for congenital lactic acidosis with 25-75 mg/kg-day DCA orally for several months had a two-fold increase in serum transaminases, suggesting preclinical hepatic toxicity. This increase was also reversible after the treatment ended. One child received oral doses of $\leq$25 mg/kg-day for five years before death from pneumonia.

Nerve conduction velocities and amplitudes were studied for one year in 27 patients with congenital lactic acidemia who received sodium dichloroacetate treatment (Spruijt et al., 2001). The patients (16 male, 11 female) whose age ranged from 9 months to 37.4 years (mean 9.8 ± 9.4 years), were started on 50 mg/kg-day DCA and were coadministered 100 mg/day thiamine. Lactate and plasma DCA concentrations were measured at 3, 6, and 12 months, and pharmacokinetics of DCA was measured at 3 and 12 months (data were not reported for these time intervals). All but two of the patients had normal baseline nerve conduction tests prior to DCA administration. Twelve of the patients (9 male, 3 female) who had prior normal baseline electrophysiology showed evidence of neuropathy (decreased nerve conduction velocity and response amplitude) by the end of treatment. Three patients showed neuropathy early, within 3 months of treatment. Neuropathy increased during treatment in the two patients who exhibited neuropathy prior to the start of therapy. Patients with neuropathy were notably older than those with normal electrophysiology; while age was significantly correlated with the deterioration in conduction of some nerves at certain time periods, there was an insufficient number of individuals in the study to provide statistical power for testing age and the deterioration of most nerves.

Data on DCA in humans are scarce, and the fact that available studies in humans have predominantly focused on individuals who were being treated for a disease complicates the assessment of DCA-mediated toxicity. Many of these individuals were extremely ill and the fact that they were being dosed with other medications in addition to DCA presents the possibility that any adverse effects of DCA treatment might not be observed by a clinician. For example, effects might have been masked or developed over a longer period than the treatment period used. To date, there have been no reports of DCA-induced neoplasia in any tissue or gonadal toxicity in humans.

4.2. STUDIES IN ANIMALS

4.2.1. Acute and Subchronic Studies

Mice

Male mice were administered varying levels of DCA (0.1 to 3 g/L) in their drinking water for up to 8 weeks and were subsequently examined for accumulation of glycogen in their liver (Kato-Weinstein et al., 1998). Significant increases in the glycogen content of the liver were observed after two weeks with concentrations as low as 0.5 g/L (100 mg/kg-day). Glycogen concentrations reached maximum levels within 1 week of treatment at concentrations of DCA in drinking water of 1 g/L (200 mg/kg-day) and above. The glycogen that accumulated at this early stage was subject to mobilization by fasting. However, with continued treatment, the deposited glycogen became increasingly resistant to mobilization. After approximately 8 weeks, the glycogen content of the livers of DCA-treated mice were the same for animals tested in fasted and nonfasted states.

Male $B6C3F_1$ mice (12/dose level) were exposed to dichloroacetate concentrations of 0, 0.3, 1, or 2 g/L in drinking water for 14 days (Sanchez and Bull, 1990). This corresponded to doses of approximately 0, 57, 190 or 380 mg/kg-day. Male and female Swiss-Webster mice (4/sex/dose) were exposed to 0, 1 or 2 g/L (0, 190 or 380 mg/kg-day) for 14 days. In male $B6C3F_1$ mice, exposure to 190 and 380 mg/kg-day increased the liver weight and hepatocyte diameters. Increased hepatocyte size was attributed to increased glycogen deposition. At these dosage levels, livers had pale streaks running on the surface and, occasionally, discrete round white areas. In Swiss-Webster mice, liver weight increased at the high dose level in both sexes and the relative liver-to-body weight ratio increased in both sexes in a dose-related manner. Localized areas of necrosis were observed at both doses. A significant increase in the labeling index of hepatocytes was observed in male $B6C3F_1$ mice treated with 2 g/L at day 14, but not at lower doses. These observations led the authors to speculate that the carcinogenic effects of DCA seen in other studies (Bull et al., 1990) may be related to DNA damage and increased repair activities, and abnormal glycogen deposition may be an underlying mechanism of DCA-induced hepatotoxicity. Based on histological evidence of liver toxicity the study identifies a NOAEL of 57 mg/kg-day for $B6C3F_1$ mice and a LOAEL of 190 mg/kg-day for $B6C3F_1$ and Swiss-Webster mice.

Rats

In an acute study investigating DCA-induced metabolic changes, male Sprague-Dawley rats were administered a single 100 mg/kg dose of DCA by gavage (Evans and Stacpoole, 1982). Animals were sacrificed 0.5, 1, 3, 6, 12, or 24 hours later (three animals at each time period). Blood glucose, pyruvate, and lactate were significantly decreased at 3 hours after dosing with a return to basal levels at 6 hours. No significant change in pyruvate dehydrogenase complex activation was detected in the liver. The authors did not evaluate other health effects.

The effect of multiple doses of DCA on pyruvate dehydrogenase complex activity was assessed by the administration of three successive 100 mg/kg doses at 6-hour intervals to male Sprague-Dawley rats. Groups of three animals were sacrificed 3, 6, 12, 24, 48, and 72 hours after dosing. Multiple dosing resulted in a progressive rise in pyruvate dehydrogenase complex activity with each dose. Activity was determined as the ratio of active to total ($CaCl_2$- and $MgCl_2$-activated) pyruvate dehydrogenase complex. Activity returned to basal levels 24 hours after the second and third dose (Evans and Stacpoole, 1982).

In a third experiment, adult Sprague-Dawley rats were exposed to 100 mg/kg-day DCA by gastric intubation for 7 days. Blood lactate was decreased, and the reduced level was maintained until 48 hours after the final dose. The activity of pyruvate dehydrogenase significantly increased in muscle and liver tissue, but returned to basal levels within 24 hours after cessation of dosing.

Davis (1990) performed a similar study of DCA-induced metabolic changes in rats, but used slightly larger dose groups and two dose levels of DCA. Groups of Sprague-Dawley rats (5/sex/dosage group) were administered a single dose of DCA by gavage (three times in one day) for a total dose of 0, 120, or 316 mg/kg-day. Animals were subsequently examined for alterations in glucose and lactate levels in the plasma, liver and kidney. Decreased plasma lactate levels were observed in both sexes in both dosage groups. Plasma glucose levels were not decreased and, although tissue lactate levels were reduced, the differences were not significantly different from the controls.

In an earlier study, Davis (1986) evaluated the administration of DCA in drinking water for a two-week period on metabolism in the rat. Sprague-Dawley rats (5/sex/group) were given

water containing 0, 30, 125, 500, or 1,875 mg/L of DCA for 14 days. These concentrations correspond to target dose levels of 0, 10, 40, 150, or 600 mg/kg-day. Rats in the high-dose group lost weight during the first week and then began gaining weight normally during the second week. This effect was not statistically significant and was correlated with decreased water and food consumption in the high-dose group. Urine volume and osmolarity were not significantly affected in any groups; however, there was a trend toward decreased volume and increased osmolarity with increased DCA exposure (consistent with decreased water consumption). Ammonia excretion and renal phosphate-dependent glutaminase activity tended to increase with increasing exposure. These effects were considered by the author to be normal adaptation to an acid load. Lactate and pyruvate levels in females were not significantly affected in either the liver or kidney, although a trend toward decreased liver lactate was observed. Blood glucose levels were not significantly affected in either males or females. The study identified a NOAEL in rats of 150 mg/kg-day.

In a subchronic study, the metabolic and toxic effects of DCA were investigated in rats following dietary administration of the compound (Yount et al., 1982). Doses varied from 4 mmol/kg-day at the beginning of the study to 2.5 mmol/kg-day (516 to 323 mg/kg-day) during the 12-week study period. Dichloroacetic acid did not affect plasma glucose levels, but led to decreased plasma triacylglycerol and increased plasma ketone bodies. Hind limb weakness and abnormal gait were observed in exposed animals within 2 to 4 weeks, while decreased nerve conduction velocities was observed in sural, tibial, and motor nerves. Decreased food consumption and decreased weight gain occurred in exposed animals, and organ-to-body weight ratios were increased for the adrenal glands, brain, and kidney. Dichloroacetic acid also caused hepatomegaly and there was evidence of testicular degeneration.

Groups of five male Sprague-Dawley rats were administered DCA (0 or 1,100 mg/kg-day) in drinking water for 90 days (Bhat et al., 1991). Body weights were monitored throughout the study. Following the 90-day treatment regimen, the animals were sacrificed and selected organs were isolated for evaluation. The following organs were weighed and examined for histopathological alterations: liver, lung, heart, spleen, thymus, kidney, testes, and pancreas. The brain and liver were also examined for collagen deposition. At sacrifice, the average body weight of the DCA-treated group was 66% that of the control group. The DCA-treated animals also had increased liver weight ($p<0.01$), increased liver-to-body weight ratios ($p<0.01$), and increased liver collagen deposition compared to control animals. Perivascular inflammation was

noted in the lungs and focal vacuolation and gliosis were present in the forebrain and brain stem of the DCA-treated group. The authors reported a progressive DCA-related decrease in water intake (presumably taste aversion) with a concomitant drop in food consumption. This potential confounder could be the underlying cause for the observed weight loss in the treated animals and could have contributed to other reported effects of DCA including liver alterations. The examination of a single high-dose limits the study.

Mather et al. (1990) administered Sprague-Dawley rats (10 males/dosage group) DCA in their drinking water at 0, 0.05, 0.5, or 5 g/L (equivalent to dosage levels of approximately 0, 3.9, 35.5, or 345 mg/kg-day, respectively) for 90 days. Water consumption was significantly ($p<0.05$) reduced in the 0.5 and 5 g/L treatment groups when measured at two months of exposure. Terminal body weights were significantly reduced ($p<0.05$), and there were increases ($p<0.05$) in liver- and kidney-to-body weight ratios at dose levels of 35.5 mg/kg-day or greater. At the highest dose, the spleen-to-body weight ratio increased. Total serum protein levels were significantly depressed at all doses. Significant increases in alkaline phosphatase were seen at the two highest doses, while alanine aminotransferase levels were increased at the highest dose. Hepatic peroxisomal beta-oxidation activity was significantly increased at the highest dose (as measured by [^{14}C] palmitoyl-CoA oxidation), but no effects were seen on hepatic microsomal enzyme activity. Liver effects were also observed at the high dose, including focal hepatocellular enlargement, intracellular swelling, and glycogen accumulation. Kidney effects at the highest dose used were characterized by diffuse degeneration of the tubular epithelium and glomeruli. Although spleen weights increased, histopathological changes in the spleen were not observed. No consistent effects were observed for immunological parameters, such as antibody production, delayed hypersensitivity, natural killer cell cytotoxicity, or production of PGE_2 or IL-2. Based on hepatic and renal effects in male rats, this study defined a NOAEL and a LOAEL of 3.9 and 35.5 mg/kg-day, respectively.

Katz et al. (1981) evaluated the effect of DCA in rats following a 3-month exposure and a postexposure recovery period. Sodium dichloroacetate was administered to Sprague-Dawley rats (10 to 15/sex/group) by gavage at dose levels of 0, 125, 500, or 2,000 mg/kg-day for 3 months. Five more rats per sex were added to the control group and the high-dose group. They were monitored for an additional 4 weeks after the 3-month feeding period was discontinued. Two rats of each sex in the 2,000 mg/kg-day group died during the study. The major signs of intoxication were hind limb paralysis and frequent urination. Two rats (one of each sex)

exhibiting these signs appeared to recover completely during the 4-week recovery period. Body weight gain was significantly depressed in a dose-dependent manner at all dose levels during the dosing period. Minimal effects on hematological parameters were observed at the two highest doses. All groups exhibited significant depressions in blood glucose and lactate, while creatinine levels increased. Male rats exhibited significantly depressed blood levels of total protein, triglycerides, iron, and calcium, as well as elevated levels of total and direct bilirubin, sodium, and potassium. Cessation of treatment was followed by a return to baseline levels in all parameters. The mean relative weights of the liver, kidneys, and adrenal glands were significantly increased in a dose-dependent fashion, but both absolute and relative organ weights tended to approach those of the controls during the 4-week recovery period. The brain and testes were the target organs of DCA intoxication. Brain lesions (characterized by vacuolization of the myelinated white tracts) were observed in the cerebrum and cerebellum of treated rats of both sexes in all dose groups (combined incidence rates of 60% at 125 mg/kg-day and 100% at 500 and 2,000 mg/kg-day). In 3/8 rats, the brain lesions persisted after cessation of treatment. Based on these effects on organ weights and brain lesions, this study identified a LOAEL of 125 mg/kg-day, the lowest dose tested.

Moser et al. (1999) extended the evaluation of the neurotoxic effects of DCA exposure in a series of experiments in weanling and adult rats. The study used a neurobehavioral screening battery under varying exposure durations (acute, subchronic, and chronic) and routes of administration (oral gavage and drinking water). The following is a description of the subchronic study which consisted of several experiments (designated by the authors as experiments 2, 4, 5a, 5b, 6a, 6b, and 7a and 7b). None of the experiments employed a control group. Experiments 2, 7a and 7b examined adult rats, while experiments 4, 5a, 5b, 6a, and 6b were conducted on weanling rats.

In experiment 2, Long-Evans (LE) rats (80-days-old) were treated with 30, 100, 300, or 1,000 mg/kg-day by oral gavage for 5 d/wk for 10 weeks, with a 1-week recovery period. The results revealed alterations in the gait of adult rats in the 300 and 1,000 mg/kg-day dose groups. In addition, mild tremor, hypotonia, and decreased forelimb grip strength was observed at the high dose. The 100 mg/kg-day dose was a NOAEL.

In experiments 7a and 7b, LE and F344 rats (68-69-days-old) were administered DCA (via drinking water) at doses of 23, 122, or 220 mg/kg-day (LE rats) or 18, 91, or 167 mg/kg-day (F344 rats) for 8 weeks, plus 2-week recovery period. Some of the F344 rats in the low-dose

group showed gait abnormalities. Gait abnormalities and decreased forelimb and hind limb grip strength were noted in the mid- and high-dose LE and F344 rats. In addition, increased foot splay was noted in the F344 rats. A chest-clasping response was seen in the high-dose F344 rats. With the exception of gait deficit and decreased hind limb grip strength, both strains showed recovery 2 weeks after exposure was discontinued. In F344 rats, the low dose of 18 mg/kg-day was a LOAEL for gait abnormalities. In LE rats, 23 mg/kg-day was a NOAEL and the LOAEL was 122 mg/kg-day.

Experiment 4 involved exposure of weanling F344 rats (30-days-old) to 162 or 308 mg/kg-day DCA in the drinking water for 12 weeks, plus a 5-week recovery period. Exposure to the high dose was discontinued at 3 weeks due to severe toxicity; the time-weighted intake for this dose group was 308 mg/kg-day. The low-dose group was exposed to DCA for 12 weeks as originally planned. The high-dose animals exhibited gait abnormalities that were still evident 14 weeks after exposure ended. The high-dose animals also displayed decreased hind limb grip strength, decreased forelimb grip strength, altered righting reflex, and lowered motor activity. Although more pronounced in the high-dose animals, these effects were also observed in the low-dose animals, with peak effects evident during the 9th and 12th week of exposure. A dose of 162 mg/kg-day was a LOAEL for neurotoxic effects in weanling F344 rats.

Experiment 5 included two segments (5a and 5b) and was intended to compare the potency of DCA in drinking water and by oral gavage. In experiment 5a, weanling F344 rats (28-29-days-old) were exposed to drinking water containing 16, 66, or 172 mg/kg-day DCA for 12 weeks, plus a 15-week recovery period. Another group of weanling F344 rats (experiment 5b) were treated by gavage to 176 mg/kg-day DCA for 12 weeks, plus a 15-week recovery period. Clear signs of neurotoxicity were observed in high-dose (172 mg/kg-day) weanling rats of experiment 5a (drinking water route). Neurotoxic signs consisted of gait abnormalities, righting reflex deficits, decreased motor activity, decreased grip strength, and tremors. Progressive gait changes and decreased motor activity were evident in the mid-dose animals (66 mg/kg-day). Low-dose (16 mg/kg-day) animals exhibited moderate effects on gait. In gavage-dosed rats (176 mg/kg-day; experiment 5b), gait abnormalities developed within 3 weeks and became progressively worse during dosing. In contrast to the drinking water route, hind limb grip strength and other neuromuscular endpoints were not affected by gavage treatment.

Experiment 6 involved the exposure of weanling LE rats (experiment 6a) or weanling F344 rats (experiment 6b) to drinking water containing DCA at dose levels of 17, 88 or 192

mg/kg-day (experiment 6a) or 16, 89, 173 mg/kg-day (experiment 6b) for 13 weeks. The purpose of experiment 6 was to investigate potential strain differences in the response to DCA. Changes were assessed using a functional observation test battery and monitoring of motor activity. The results of experiment 6 revealed that both rat strains showed progressive changes in gait in all treated groups (LOAEL, 17 mg/kg-day in LE rats and 16 mg/kg-day in F344 rats). The effect was most pronounced in the high-dose F344 rats. Hind limb grip strength was decreased throughout exposure in the mid- and high-dose LE rats (no dose-response relationship was apparent) and in the high-dose F344 rats. The effect was more pronounced in the high-dose F344 rats. Other effects at the high-dose in both strains included tremor, hypotonia and inhibition of pupil reflex. The study authors indicated that F344 rats, but not the LE strain, showed a progressive decrease in motor activity, righting deficits, and forelimb grip strength, and an increase in foot splay. Data were presented only for forelimb grip strength which was slightly decreased (<5%) at the mid-dose; the decrease was more pronounced (approximately 20%) at the high dose.

Results of the study indicated that DCA is a more potent neurotoxicant when administered to adult rats via drinking water rather than by gavage. The results also revealed that gait abnormality is a critical effect for DCA. The effect was observed at doses as low as 16 mg/kg-day (in the absence of other neuromuscular changes) and was persistent in adult rats of both strains at doses ≥91 mg/kg-day (F344) even following a 2-week recovery period. The data are consistent with the persistent histological effects in the rat cerebrum observed by Katz et al. (1981) at doses ≥125 mg/kg-day. The data also revealed that hind limbs may be preferentially affected by DCA.

Data from experiment 5 demonstrated partial recovery of neurotoxic effects, e.g., following a 13-week intake of 172 mg/kg-day. Experiments 6 and 7 illustrate that F344 rats are more sensitive than the LE rat strain to DCA. In regard to age differences, limited results show that the severity of neuromuscular toxicity was somewhat greater in rats when exposures began shortly after weaning.

Dogs

Three studies have been performed in dogs. The first (Ribes et al., 1979) reported decreased lactate and pyruvate levels persisting for 48 hours (35 and 27% of basal values, respectively) following a single oral dose of 150 mg/kg of sodium dichloroacetate. Blood glucose levels were unchanged for the first 4 hours postdosing, but declined significantly ($p<0.01$) at 24-28 hours and then returned to their initial levels at the end of the 48-hour study. Longer DCA administration (150 mg/kg-day for 7 days) caused decreases in blood glucose, lactate, pyruvate, cholesterol, and oxaloacetate concentrations. All serum values returned to their initial values within 2-6 days following treatment. Ketone bodies were not reported in the urine.

Katz et al. (1981) studied the subchronic administration of sodium dichloroacetate (0, 50, 75, or 100 mg/kg-day by capsule for 13 weeks) to four beagle dogs/sex in the control and high-dose groups and three dogs/sex in the other groups. Female dogs at all doses showed markedly reduced appetites and both sexes exhibited dose-dependent weight losses, which were reversed after the treatment ended. One female at 75 mg/kg-day died on day 40, and one at 100 mg/kg-day died on day 88. The animals exhibited anorexia, ataxia, hind limb weakness, and reduced activity. Bloody stools, vomiting, and paralysis were also observed at the highest dose level. Dose-related decreases in erythrocyte counts, hematocrits, and hemoglobin levels were reported. Mean blood glucose, lactate, and pyruvate levels were significantly decreased in all treated animals. The parameters returned to normal in those animals monitored following treatment. Treated dogs also exhibited lung consolidation. Histopathology showed neurological effects (slight to moderate vacuolization of white myelinated tracts in the cerebrum and cerebellum), and liver and gall bladder effects (an increased incidence of hemosiderin-laden Kupffer cells in the liver and cystic mucosal hyperplasia in the gall bladder); these effects were persistent through the 5-week recovery period. Indirect effects including increased incidence and/or severity of pulmonary inflammatory lesions were also attributed to DCA treatment. Based on the study results, the lowest dose of 50 mg/kg-day was identified as a LOAEL.

Slightly lower doses were used in a separate subchronic study (Cicmanec et al., 1991), in which juvenile beagle dogs (4-months-old; 5/sex/dose) received daily oral doses of 0, 12.5, 39.5, or 72 mg/kg-day DCA in gelatin capsules for 90 days. At study termination, organ weights were determined and tissues were examined microscopically. Overt clinical signs were evident in the high-dose animals throughout the duration of the experiment. Dyspnea (shortness of breath or

difficulty in breathing) was observed in high-dose animals starting at day 45, and worsened with time. Partial paralysis of the hind limbs was observed in three animals in the high-dose group during the latter half of the exposure period. Conjunctivitis was observed in 24/30 treated animals and a few controls during the first month, and became more severe later in the study. The occurrence of ocular effects appeared to be dose-related, with 8/10 high-dose dogs affected. Reduction of food and water intake was noted in DCA-treated dogs, although the effect did not appear to be dose-related. High-dose males exhibited a 16% reduction in body weight, while high-dose females and mid-dose males experienced a 9% reduction in weight gain over the duration of the study. Mid-dose females exhibited an 11% reduction in weight gain. Dogs in the mid- and high-dose groups experienced sporadic diarrhea. The most severely affected dogs required fluid therapy to prevent severe dehydration. One female and two males treated at 72 mg/kg-day died during the study. These deaths were attributed to pneumonia and dehydration.

Statistically significant decreases in erythrocyte count and hemoglobin levels were observed in high-dose dogs at day 30. Trend analysis of serum biochemistry data indicated apparent increases in lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase activity in the high-dose groups at some time points. These findings were consistent with microscopic tissue observations. Relative liver weight was significantly increased in all dose groups, and absolute liver weight was increased in all but high-dose males. Pathological examination revealed multiple changes in the organs of animals treated with DCA, including: mild vacuolar change (most prevalent at the low dose), inflammation, and hemosiderosis in the liver; and chronic inflammation and acinar degeneration in the pancreas. While the primary lesions included pale and discolored kidneys, the severity of these lesions was ranked as mild or moderate. Microscopic examination of the brain revealed mild vacuolization of white myelinated tracts in the cerebrum and/or cerebellum of some animals in low-, mid-, and high-dose DCA treatment groups. Mild vacuolar change was noted in the medulla and spinal cord of some males, while mild meningoencephalitis was present in one high-dose female. Microscopic testicular lesions were also noted in treated dogs, and are further discussed in Section 4.3. A LOAEL of 12.5 mg/kg-day can be identified, based on visual organ effects (neurological changes, hepatic vacuolization, and testicular effects) and increased liver weights.

4.2.2. Chronic Studies and Cancer Bioassays

Mice

In one of the earliest studies of DCA tumorigenesis in mice, Herren-Freund et al.(1987) gave male B6C3F_1 mice (28-days-old) drinking water containing 0, 2, or 5 g/L of DCA (corresponding to about 0, 400, or 1,000 mg/kg-day). The 400 and 1000 mg/kg-day groups were also pretreated with ethylnitrosourea (ENU). An additional high-dose group (1,000 mg/kg-day) did not receive ENU. Animals were sacrificed after 61 weeks of exposure and examined for tumors. In the control group (not exposed to either ENU or DCA), there were no hepatocellular carcinomas. In mice pretreated with ENU and subsequently administered 400 or 1,000 mg/kg-day DCA, the incidence of hepatocellular carcinomas was 66 and 78%, respectively. In the 1,000 mg/kg-day dose group treated only with DCA, hepatocellular carcinoma incidence was 81%; this result prompted the authors to conclude that DCA was carcinogenic at this dose in the absence of initiation.

Bull et al. (1990) used a stop-dosing regimen to evaluate the time required to onset liver tumors in dosed mice. Groups of B6C3F_1 mice were provided drinking water containing DCA at concentrations of 0 mg/L (35 males, 10 females), or 1 g/L (11 males) for 52 weeks, or 2 g/L (11 males) for 37 weeks with a 15-week recovery period, or 2 g/L (24 males, 10 females) for 52 weeks. Based on the authors' graphical data for total dose, mean intake rates were approximately 140 mg/kg-day (52 weeks) at the low dose and 280 mg/kg-day (37 weeks) or 300 mg/kg-day (52 weeks) at the high dose. Although treatment did not affect survival or body weight, increased hepatic lesions were observed in all low- and high-dose groups, including: increased absolute and relative liver weights, cytomegaly, massive accumulation of glycogen in hepatocytes, and foci of necrosis or basophilic cellular alteration. The LOAEL for chronic non-neoplastic effects established by this study was approximately 140 mg/kg-day for 52 weeks.

At sacrifice, no liver tumors were reported in the female mouse group, but hyperplastic nodules were observed microscopically in the livers of 3/10 treated animals (Bull et al., 1990). In the male mouse group exposed to 140 mg/kg-day for 52 weeks, a total of 3 hepatic lesions were noted in 2 of 11 mice; the single lesion examined histologically was a hyperplastic nodule. Of the 24 male mice exposed to 300 mg/kg-day for 52 weeks, 92 hepatic lesions were scored in 23 mice; of the 23 lesions in 10 mice that were examined histologically, 15 lesions in 9 mice were hyperplastic nodules, 2 lesions in 2 mice were adenomas, and 6 lesions in 5 mice were

hepatocellular carcinomas. Finally, of the 11 male mice exposed to 280 mg/kg-day for 37 weeks, 23 hepatic lesions were found in 7 mice; of the 19 lesions in 7 mice that were examined histologically, 15 lesions in 6 mice were hyperplastic nodules, 2 mice had an adenoma, and no hepatocarcinomas were observed. The authors concluded that tumorigenesis by DCA may depend largely on stimulation of cell division secondary to hepatotoxic damage.

The U.S. EPA (1991b) evaluated the carcinogenicity of DCA in female B6C3F_1 mice. Following exposures to 0, 0.5, or 3.5 g/L DCA (approximately 0, 80 or 400 mg/kg-day) in drinking water for 104 weeks, the high-dose group had a 100% hepatocellular tumor incidence and a tumor multiplicity of 8.36 tumors/animal. Mice receiving 0.5 g/L DCA had a tumor incidence of 20% and a tumor multiplicity of 0.2 tumors/animal. The untreated control group had an incidence of 7.7% and a multiplicity of 0.1 tumors/animal.

DeAngelo et al. (1991) evaluated differential exposure doses and exposure durations on the development of tumors in male mice. Dichloroacetic acid was administered to B6C3F_1 mice (50 males/dosage group) in their drinking water at concentrations of 0, 0.05, 0.5, 3.5, or 5.0 g/L for 60 weeks. These doses correspond to levels of 0, 7.6, 77, 410, and 486 mg/kg-day. Other groups of mice were administered DCA at 7.6 or 77 mg/kg-day for 75 weeks. In high-dose treated mice, water consumption was reduced to 60% of controls. Body weight was decreased at the two highest dose levels, and relative liver weight was increased at the three highest dose levels. An increase in kidney weight was seen only at 410 mg/kg-day. No effects were seen on testes or spleen weight. Therefore, the LOAEL for increased relative liver weight was 77 mg/kg-day for the 60-week study, and the NOAEL was 7.6 mg/kg-day. At 75 weeks, the relative liver weight for the 77 mg/kg-day dose was increased, but the difference from controls was not statistically significant. In mice receiving 410 mg/kg-day, 58% had hyperplastic nodules, 100% had hepatocellular adenomas and 67% had hepatocellular carcinomas. At the higher dose level of 486 mg/kg-day, 83% of the mice had hyperplastic nodules, 80% had hepatocellular adenomas, and 83% had hepatocellular carcinomas. Incidences in other groups (7.6 and 77 mg/kg-day) were similar to controls.

In a limited-dose cancer study, Daniel et al. (1992) exposed B6C3F_1 male mice (33/dose level) to DCA in drinking water at concentrations of 0 or 0.5 g/L (0 or 88 mg/kg-day, mean weighted average) for 104 weeks. At terminal sacrifice, absolute and relative liver weights were significantly increased ($p<0.01$) when compared to untreated controls. The mean daily water

consumption was not significantly reduced (6.1 vs. 6.2 mL/mouse/day). There was an increased incidence of hepatocellular necrosis, chronic inflammation, and cytomegaly in the treated group when compared to controls. No significant changes were found in other organ weights (kidney, testes, and spleen), body weights, or survival in the treated groups when compared to untreated controls. A LOAEL for nonneoplastic hepatic effects in this study was 88 mg/kg-day.

Hepatocellular carcinomas (15/24 or 63% versus 2/20 or 10% in controls) and hepatocellular adenomas (10/24 or 42% versus 1/20 or 5%) increased in animals that survived 104 weeks to terminal sacrifice (Daniel et al., 1992). The increase in the number of hyperplastic nodules observed in treated animals (2/24 or 8%, versus 0 in controls) was not statistically significant. No adenomas or nodules were found at the 30-week interim necropsies and there was no interim sacrifice at 60 weeks which might have provided data on whether or not hyperplastic nodules had started to form.

Two recent studies evaluated the carcinogenic response of DCA exposure in the female mouse. In the first study, female B6C3F_1 mice were administered 2.0, 6.67, or 20.0 mmol/L DCA in drinking water (40, 115, or 330 mg/kg-day) from 7 to 8 weeks of age to sacrifice at 360 or 576 days (~ 51 or 82 weeks) of exposure (Pereira and Phelps, 1996). Significant increases in the percentage of animals with altered hepatocyte foci and liver adenomas were seen in the 115 and 330 mg/kg-day groups, including: after 51 weeks, 40.0% with foci and 35% with adenomas at 330 mg/kg-day; after 82 weeks, 39.3% with foci and 25% with adenomas at 115 mg/kg-day, and 89.5% with foci and 84.2% with adenomas at 330 mg/kg-day. A significant increase in the percentage (26.3%) of animals with liver carcinomas was only seen in the 330 mg/kg-day group after 82 weeks of exposure. The authors concluded that the relationship of altered hepatocyte foci frequency, hepatocellular adenoma occurrence, and hepatocellular carcinoma occurrence to DCA concentration were best described by second-order regression.

In the second study, liver tumors were initiated in female B6C3F_1 mice with 25 mg/kg methylnitrosourea (MNU); the mice were then administered 2.0, 6.67, or 20.0 mmol/L DCA in their drinking water (50, 167, or 468 mg/kg-day) from age 7 weeks to sacrifice 31 or 52 weeks later to characterize tumor promotion by DCA (Pereira and Phelps, 1996). A 4 mL/kg sterile saline vehicle control was included in the study. Significant increases in the percentage of animals with liver adenomas were seen in the 468 mg/kg-day group after 31 weeks of exposure (50.0% versus 0% in control) and 52 weeks of exposure (73.1% versus 17% in control). A significant increase in the percentage of animals with altered hepatocyte foci was also seen after

31 weeks of exposure (80.0% versus 20.0% in control) and 52 weeks of exposure (50.0% versus 10.0% in control). When the exposure to 468 mg/kg-day DCA was terminated after 31 weeks it was followed by a 21-week recovery period. The authors observed decreased yield of altered hepatocytes and tumors, indicating that continued existence of these lesions was dependent on continuous exposure to DCA. The tumor-promoting activity of DCA exhibited a second-order relationship to drinking water concentration, so that a sharp rise in potency was seen between 167 and 468 mg/kg-day.

To better understand the mechanisms of dichloroacetate carcinogenicity, Stauber and Bull (1997) investigated changes in the replication and phenotype of cells from hepatic tumors. Male B6C3F_1 mice were pretreated with 2.0 g/L of dichloroacetate in drinking water for 38 or 50 weeks, respectively. The mice (12 animals/dose) were then administered drinking water containing 0, 0.02, 0.1, 0.5, 1.0, or 2.0 g/L dichloroacetate for two additional weeks. At three days prior to sacrifice, 5-bromo-2-deoxyuridine (BrdU) was administered via subcutaneously implanted pumps to label the DNA *in vivo*. The animals were sacrificed and the liver tissue was stained and examined. A transient, but significant increase in hepatocyte division rates as compared to controls was evident for the first 14 days of treatment with 2 g/L, but was not apparent at 28, 280 and 350 days of treatment. DCA-induced tumors were stained with anti-c-*Jun* and anti-c-*Fos* antibodies. Dichloroacetate-induced altered hepatic foci (AHF) and tumors were largely basophilic and reacted uniformly to antibodies against c-*Jun* and c-*Fos* (nuclear transcription factors). The c-*Jun* protein was localized in the cytoplasm and the c-*Fos* protein was found in the nucleus. The AHF and tumors that were c-*Jun* positive displayed a dose-dependent increase in cell replication during the labeling period. The cell replication rate in dichloroacetate-induced AHF and tumors were dependent on dichloroacetate treatment, but this effect was observed only in the c-*Jun* positive regions of the lesions (see Section 4.4 for additional data on the effects of DCA on transcription factors).

DeAngelo et al. (1999) reported on the carcinogenesis of DCA in male B6C3F_1 mice. The mice were exposed to 0, 0.05, 0.5, 1, 2, or 3.5 g/L of DCA in drinking water for 90-100 weeks. The exposures corresponded to mean daily doses of 0, 8, 84, 168, 315, or 429 mg/kg-day, respectively. The cumulative incidence of hepatocellular carcinomas was significantly increased in animals exposed to 1 g/L (71%), 2 g/L (95%), and 3.5 g/L (100%) when compared to control (26%) (see Table 5-8). Hepatocellular carcinoma multiplicity (tumor/animal) significantly increased in all treatment groups as follows: 0.05 g/L (0.58), 0.5 g/L (0.68), 1 g/L (1.29), 2 g/L (2.47) and 3.5 g/L (2.90) when compared to the control group (0.28). The

cumulative incidence of hepatocellular adenomas significantly increased in animals exposed to 1 g/L (51.4%), 2 g/L (42.9%), and 3.5 g/L (45%) when compared to controls (10%) and the 0.5 g/L group (20%). Hepatocellular adenoma multiplicity (tumor/animal) significantly increased in the following dose groups: 0.5 g/L (0.32), 1 g/L (0.80), 2 g/L (0.57), and 3.5 g/L (0.64) as compared to controls (0.12). By the end of the study, body weights decreased 18% in mice treated with 2 and 3.5 g/L compared to the controls. All DCA doses, except the lowest dose (0.05 g/L), resulted in an increase in the severity of hepatic necrosis compared to the controls, when measured at 26 weeks. Necrosis was mild (between 25 and 50% of the liver sections were affected) and transient at 1 and 2 g/L (severity did not increase at later time points with these doses). Hepatic peroxisome proliferation increased in the high-dose group, but did not correlate with liver tumor response. The severity of hepatotoxicity increased with DCA concentration. Below 1 g/L, hepatotoxicity was mild and transitory (as evidenced by histopathological examination and serum enzyme levels) and there was no significant increase in labeling index outside of proliferative lesions. Based on these observations, the authors concluded that DCA-induced liver cancer does not appear to be dependent upon peroxisome induction or chemically-sustained cell proliferation. Hepatotoxicity, especially at the higher doses, may exert an important influence on the carcinogenic process.

Rats

DeAngelo et al. (1996) reported the results of two studies of male Fischer 344 rats exposed to DCA in drinking water. Cancer as well as noncancer toxicity endpoints were assessed. The two studies are independent of each other (they were conducted in different laboratories with different animals) and are described separately below.

In the first study, 28-day-old male F344 rats were given drinking water containing DCA at concentrations of 0 (78/group), 0.05 (60/group), 0.5 (60/group) or 5.0 g/L (78/group). A second control group (50/group) was provided water containing 2.0 g/L NaCl. Animals were observed daily for physiological and behavioral responses and for overt signs of toxicity. Body weights and water consumption were measured throughout the study. All animals were treated for 100 weeks, except for animals in the 5.0 g/L group, which exhibited signs of peripheral neuropathy. In response to this overt toxic effect, the concentration was sequentially lowered to 2.5 g/L at 9 weeks, then 2.0 g/L at 23 weeks and finally to 1.0 g/L at 52 weeks. When the neuropathy did not reverse or diminish, the animals were sacrificed at 60 weeks and excluded from the report. Based on measured water intake in the 0, 0.05 and 0.5 g/L groups, the time-

weighted average doses were 0, 3.6, and 40.2 mg/kg-day, respectively. Interim sacrifices for each dose group were performed at 15, 30, 45, 60, and 100 weeks while the NaCl control group was sacrificed at 104 weeks. The body, liver, kidneys, testes, and spleen were weighed and examined for gross lesions at the interim sacrifices, while at the final sacrifice, a complete necropsy was performed on all animals. No differences were observed in water consumption, final body weight, absolute or relative liver weight, and kidneys or spleen weight, at dosages of 3.6 or 40.2 mg/kg-day at any time point. However, absolute and relative testicular weights were mildly, but significantly, increased at the 40.2 mg/kg-day dose at final sacrifice. Increased hepatocellular vacuolization was detected, but there was no increase in hepatocyte proliferation at any dose group. There was also a noted lack of necrosis observed in doses carried out to final sacrifice at 100 weeks.

Hepatic neoplastic lesions were examined at sacrifice (DeAngelo et al., 1996). At a dose of 40.2 mg/kg-day DCA, there was a statistically significant increase in the cumulative incidence of combined hepatocellular neoplasia (21.4% vs. 4.4%; $p<0.05$) and total proliferative lesions in the liver (34.9% vs. 8.7%; $p<0.05$) compared to controls. This was not observed at the lower dose of 3.6 mg/kg-day. Tumor multiplicity was significantly increased in the 40.2 mg/kg-day group as compared to controls. There was also a significant increase in combined hepatocellular neoplasia (0.04 vs. 0.3) and total proliferative lesions (0.41 vs. 0.09). Other tumors were not increased over control values.

In the second study by DeAngelo et al. (1996), male F344 rats were exposed to DCA concentrations of 2.5 g/L DCA in their drinking water (78/group) or to deionized water (78/group). The concentration of DCA was lowered to 2 g/L at 5 weeks, to 1.5 g/L at 8 weeks, and to 1.0 g/L at 26 weeks. This corresponded to a time-weighted average concentration of 1.6 g/L and a time-weighted average dose of 139 mg/kg-day over the 103-week exposure period. Interim sacrifices for each dose group were performed at 14, 26, 52, 78 and 103 weeks. The liver, kidneys, testes, thyroid, stomach, rectum, duodenum, ileum, jejunum, colon, urinary bladder, and spleen were examined for gross lesions at all time periods. In this study the mean final body weight of DCA-exposed animals was significantly reduced to 73% of the deionized water control group. Absolute testes weight decreased, but relative testes weight was not significantly lower than the control group. Signs of liver pathology were also minimal in this study, and this dose of DCA suppressed hepatocyte proliferation. Consistent with the first study, there was a lack of liver necrosis observed at final sacrifice.

Hepatic tumor incidence significantly increased in exposed animals compared to controls, as follows: carcinoma (21.4% vs. 3.0%, $p<0.05$), combined hepatocellular neoplasia (28.6% vs. 3.0%; $p<0.01$) and total proliferative lesions (32.1% vs. 6.1%; $p<0.01$, DeAngelo et al., 1996). Tumor multiplicity was also significantly increased in the exposed group compared to controls: combined hepatocellular neoplasia (0.36 vs. 0.03), total proliferative lesions in the liver (0.39 vs. 0.06), and carcinomas (0.25 vs. 0.03). Other tumors examined were not increased over control values.

Male Fischer 344 rats were administered time-weighted average concentrations of 0, 0.05, 0.5, or 2.4 g/L (0, 4, 40, or 296 mg/kg-day) DCA in drinking water, followed by sacrifice at intervals for up to 104 weeks by Richmond et al. (1995). No hepatoproliferative lesions were seen in the 4 mg/kg-day group, and the negative control group had only 4% hepatic adenomas. The 40 mg/kg-day group had 10% hyperplastic nodules, 21% hepatic adenomas, and 10% hepatocarcinomas after 104 weeks, while the 296 mg/kg-day group had 70% hyperplastic nodules, 26% hepatic adenomas, and 4% hepatocarcinomas after terminal sacrifice at 60 weeks. Increased numbers of altered hepatocyte foci were also seen in the 4 and 40 mg/kg-day groups, but the differences were significant only in animals from the 45-week sacrifice.

Tumor marker expression was examined in the DCA-induced hyperplastic nodules (Richmond et al., 1995). The expression of six histochemical markers of neoplastic cells (p21 *ras*, p39 c-*jun*, p55 c-*fos*, aldehyde dehydrogenase, glutathione S-transferase, and alpha fetoprotein) were examined by immunohistochemical and image analysis methods. The hyperplastic nodules were identified as having preneoplastic characteristics, while altered hepatic foci did not have preneoplastic characteristics. These observations were reported to be consistent with results obtained for DCA-induced hepatocarcinogenesis in $B6C3F_1$ mice (Daniel et al., 1992).

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

Mice

Unlike those studies in rats (discussed below), studies regarding potential reproductive effects of DCA exposure in mice have been limited to *in vitro* methodology. Dichloroacetic acid was found to inhibit *in vitro* fertilization of $B6D2F_1$ mouse gametes by Cosby and Dukelow (1992). The percent of gametes fertilized dropped from 87.0% (controls) to 67.3% or 71.8% with exposure to 100 or 1,000 mg/L DCA, respectively. A study by Hunter et al. (1996) exposed CD-1 mouse whole-embryo cultures to 0 to 14.7 mM DCA for 24 hours. The study authors found significant increases in neural-tube defects at treatment concentrations of 5.9 mM and above, heart and pharyngeal arch defects were seen at concentrations of 7.3 mM and above and eye defects, rotational defects and somite dysmorphology at concentrations of 11 mM and above.

In a follow-up study to the whole embryo culture study performed by Hunter et al. (1996), Ward et al. (2002) investigated cell-cycle disruptions in mice neurulation-stage (gd8) embryos exposed to 11 mM DCA for 6, 12, 18, or 24 hours. Dichloroacetic acid caused a slight, but not statistically-significant, increase in the number of heart cells in S phase and a slight decrease in those cells in G1 phase (measured by flow cytometry), compared to controls. Dichloroacetic acid induced a statistically-significant increase in sub-G1 events (defined as hypodiploid peaks and cells or cellular debris with less than 2n copies of DNA) in embryos incubated $\geq$12 hours, which was interpreted to be an increase in the induction of apoptosis. This effect was consistent in the head, heart, midpiece, and hindpiece regions of embryos exposed to DCA for 24 hours.

Bis I, an inhibitor of protein kinase C, did not induce sub-G1 events while staurosporine, a nonspecific protein kinase inhibitor did. When the alteration in apoptosis was analyzed using fluorescence microscopy, DCA treatment increased signals in the primordial optic tissue, the prosencephalon brain vesicle in the embryo, and the branchial arches (fetal gill-like tissue), but not the heart region. This is consistent with the cell-cycle data and the neural tube defects observed by Hunter et al. (1996). Bis I did not increase the signal in the brain region. The data suggest that DCA's inhibition of protein kinase may be a mechanism for apoptosis. However, the inhibition of protein kinase C is unlikely to be the predominant mediator of DCA-induced embryotoxicity.

Rats

Several studies have been performed to determine the potential reproductive and/or developmental toxicity of DCA exposure in rats. However, there are no single or multiple-generation studies of DCA reproductive toxicity.

The testicular toxicity of DCA was evaluated in adult male rats administered both single and multiple (up to 14 days) oral doses of 0, 18, 54, 160, 480, or 1440 mg/kg-day (Linder et al., 1997). Delayed spermiation and altered resorption of residual bodies were observed in rats given single doses of 1,500 and 3,000 mg/kg body weight; these effects persisted to varying degrees on posttreatment days 2, 14, and 28. Delayed spermiation and formation of atypical residual bodies also were observed on days 2, 5, 9, and 14 in rats dosed daily with 1,440, 480, 160 or 54 mg/kg-day, respectively. Distorted sperm heads and acrosomes were observed in step 15 spermatids after doses of 480 and 1,440 mg/kg-day for 14 days. Decreases in the percentage of motile sperm occurred after 9 days at doses of 480 and 1440 mg/kg-day, and after 14 days at 160 mg/kg-day. Increased numbers of fused epididymal sperm were observed on days 5, 9, and 14 in rats dosed with 1440, 480 and 160 mg/kg-day, respectively; other morphologic abnormalities occurred at 160 mg/kg-day and higher. On day 14, a significant decrease in epididymal weight was observed at 480 and 1,440 mg/kg-day, and epididymal sperm count was decreased at 160 mg/kg-day and higher (see also Table 5-6).

Limited, but significant, reproductive toxicity was reported by Bhat et al. (1991) following the subchronic oral dosing of DCA in male rats. Groups of male Sprague-Dawley rats (5/group) were administered 0 or 1,100 mg/kg-day DCA in drinking water for 90 days. Body weights were monitored throughout the study. The animals were sacrificed at 90 days, and selected organs, including the testes, were isolated for evaluation. Dichloroacetic acid exposure decreased testis weight ($p<0.01$) and was associated with signs of tissue atrophy. In addition, the seminiferous tubules contained very few spermatocytes, and no mature spermatozoa.

In a subchronic toxicity study, Katz et al. (1981) dosed rats (10 to 15/sex/dosage group) with 0, 125, 500, or 2,000 mg/kg-day of sodium dichloroacetate by gavage daily for 3 months. Mammary glands, prostate glands, testes with epididymis, ovaries, and uterine horns were among the large number of tissues examined for histopathological changes. Testicular germinal epithelial degeneration was seen in 40% of males at 500 mg/kg-day and in all males at 2,000

mg/kg-day. In all males at 2,000 mg/kg-day, the testes appeared aspermatogenic and contained syncytial giant cells in the germinal epithelium, while the epididymis ducts were devoid of spermatozoa. Syncytial giant cells in the germinal epithelial were seen in 20% of the male rats dosed at 500 mg/kg-day. No other effects were noted at the 125 or 500 mg/kg-day dose levels. No effects were noted in the reproductive tissues of female rats. Five rats of each sex that had received the highest dose were maintained on a normal control diet for 5 weeks after dichloroacetate treatment had been discontinued. In some of the male rats, there was evidence of germinal epithelium regeneration (50%) and spermatogenesis (25%).

Toth et al. (1992) also studied the potential reproductive effects in male rats following subchronic oral exposure to DCA using lower doses than the earlier studies. Male Long-Evans rats (18 to 19/dose) were administered 0, 31.25, 62.5, or 125 mg/kg-day sodium dichloroacetate for 10 weeks by oral gavage. Reduced final animal weights relative to controls were observed at the mid- and high-dose groups. At 31.25 mg/kg-day NaDCA and higher, relative liver weights increased, while relative kidney and spleen weights and absolute liver weights were increased at 62.5 and 125 mg/kg-day NaDCA. Significant ($p \leq 0.05$) reductions in the absolute weight of the preputial gland and epididymis were noted at all dose levels, but the absolute weight of the testis was not affected at any dose. At the two higher doses (62.5 and 125 mg/kg-day), there were significant ($p \leq 0.05$) reductions in the percentage of motile sperm, effects on sperm motion (i.e., velocity, linearity, amplitude of lateral head displacement) and reduced epididymis sperm head counts. At 125 mg/kg-day, animals also had reduced accessory organ (prostate and seminal vesicle) weights and increased relative testis weights. Histological examination of testis cross sections did not reveal any gross lesions at any dose, and cellular structures in the epididymis epithelium appeared normal. Impaired spermiation was noted in 4 of the 10 mid-dose (62.5 mg/kg-day) animals and 9 of the 10 high-dose (125 mg/kg-day) animals, and was attributed to the retention of late-step spermatids in the seminiferous tubules, as observed histologically. This finding corroborated the observed reductions in epididymal, but not testicular late-step spermatid head counts. The fertility of treated males, although reduced in the high-dose group, did not differ significantly from controls at any dose level. Based on the organ weight changes reported for the preputial gland and epididymis, as well as impaired sperm formation, a LOAEL of 31.25 mg/kg-day was identified (see also Table 5-6).

Epstein et al. (1992) investigated the time-sensitivity of DCA dosing on the development of the fetal rat. Pregnant Long-Evans rats were exposed, via oral intubation, to DCA as follows: 1,900 mg/kg-day on consecutive gestation days 6 to 8, 9 to 11, or 12 to 15; single doses of 2,400

mg/kg-day on gestation day 10, 11, 12, or 13; or single doses of 3,500 mg/kg-day on gestation day 9, 10, 11, 12, or 13. No treatment effects on maternal body or organ weights were observed. Within the 1,900 mg/kg-day exposure group, reduced mean fetal body weight was observed for days 6 to 8, and increased cardiac malformations for days 9 to 11 and 12 to 15. Single gestation day exposures increased the incidence of cardiac defects (2.5 to 3.3% and 2.9 to 3.6% at the 2,400 and 3,500 mg/kg-day doses, respectively). Collectively, these studies indicate a developmental LOAEL of 1,900 mg/kg-day.

Smith et al. (1992) performed a similar investigation into the developmental toxicity of ingested DCA when administered during organogenesis in the pregnant rat. Pregnant Long-Evans rats (19-21/group) were treated by oral intubation with 0, 900, 1,400, 1,900, or 2,400 mg/kg-day DCA on gestational days 6 to 15. Eight dams in the three high-dose groups died during treatment, appearing anorexic and sluggish prior to death; one death was determined to be accidental. Maternal weight gain, adjusted for gravid uterine weight, was significantly decreased to approximately 60% of the control value in all treatment groups. The absolute liver, spleen, and kidney weights significantly ($p \leq 0.05$) increased (approximately 13 to 19%, 16 to 28%, and 12 to 18%, respectively, compared to the control) in all dose groups, with corresponding hypertrophy in these organs. The mean percentage of resorbed implants per litter was significantly elevated in all treated dose-groups. The number of live fetuses/litter was significantly reduced by 27% at 2,400 mg/kg-day. All dose groups exhibited significant, but relatively small, dose-dependent reductions in fetal weight (approximately 89% of control group) and fetal crown-rump length (75 to 86% of the control group). There was a significant increased incidence (dose-dependent) of soft tissue and cardiovascular anomalies in all treatment groups, and of external malformations beginning at the 1,400 mg/kg-day group. No skeletal malformations were observed.

In a second experiment by Smith et al. (1992), pregnant Long-Evans rats (19-20/group) were administered 0, 14, 140, or 400 mg/kg-day DCA by gavage on gestational days 6 to 15. A significant decrease in maternal weight gain, adjusted for gravid uterine weight, was found in the mid- and high-dose dams (63 and 77% of control, respectively), as well as an increase in spleen and kidney weights at the highest dose. Absolute liver weight was significantly elevated for all dose groups compared to the control group, with 3, 8, and 14% increases observed, respectively. Dose-related hypertrophy in the liver, spleen and kidneys was reported in the two high-dose groups (no incidence data). Reduced fetal crown-rump length (5% decrease) and fetal body weight (7% decrease) were significant in the high-dose group. A dose-related increase in soft

tissue anomalies, primarily cardiovascular, was reported in the 140 and 400 mg/kg-day groups. The increase in soft tissue abnormalities was significant for the two highest dose groups and the cardiac abnormalities for the highest dose. An intraventricular septal defect between the ascending aorta and the right ventricle was most commonly observed with less frequent urogenital defects (bilateral hydronephrosis and renal papilla) and defects of the orbit also reported. Collectively, these studies determined a NOAEL of 14 mg/kg-day and a LOAEL of 140 mg/kg-day DCA for developmental effects (soft tissue anomalies) and maternal effects (reduced body weight and organ hypertrophy).

Moser et al. (1999) investigated the chronic-duration neurotoxic effects of DCA in weanling rats. Rats were exposed via drinking water to 2.5 or 3.5 g/L DCA for 24 months. However, exposures to the high dose were discontinued before the study ended because of excessive toxicity. In addition, the low dose was decreased at 6 weeks and at 10 weeks. Estimated intake levels over the exposure period were 235 mg/kg-day (for 6 months) and 137 mg/kg-day (for 24 months) for the high- and low-dose groups, respectively. Severe gait abnormalities, decreased hind limb grip strength, righting deficits, and tremors (>50% incidence) were evident in both dose groups throughout the 2-year period. Recovery was not evident even 18 months after exposure ended in the high-dose group. Treated rats also showed decreased forelimb grip strength, chest clasp, and an inhibited pupil response.

The potential developmental toxicity of DCA was studied *in vitro* using a rat whole embryo culture system (Saillenfait et al., 1995). Groups of 10 to 20 explanted embryos from Sprague-Dawley rats were cultured for 46 hours in 0, 1.0, 2.5, 3.5, 5.0, 7.5, or 10 mM DCA. A significant, dose-dependent decrease in crown rump length was seen at 3.5 mM and above, while significant, dose-related decreases in yolk sac diameter, head length, somite (embryonic segment) number, protein content, and DNA content were seen at 2.5 mM and above. In addition, several defects which were nonexistent in the 0 and 1.0 mM groups were present to a substantial degree in the higher dose groups. At 2.5 mM, 30% of the embryos had brain defects, 45% had eye defects, and 10% had reduced embryonic axis. At 3.5 mM, 95% had brain defects, 75% had eye defects, 80% had reduced embryonic axis, 15% had reduced first branchial arch, 40% had otic system defects, and 15% had defective flexion. The results indicated a teratogenic effect from DCA in this system.

Dogs

Two subchronic studies in the beagle dog examined endpoints relevant to reproductive toxicity. In the first study (Katz et al., 1981), male and female beagle dogs (3 to 4 animals/sex/dosage group) were administered oral doses of 0, 50, 75, or 100 mg/kg-day of sodium dichloroacetate by gavage for 13 weeks. Prostate gland atrophy and testicular changes (degeneration of germinal epithelium, vacuolation of Leydig cells, formation of syncytial giant cells) were observed in all treated males. These effects were qualitatively judged by the authors to be dose-dependent (no data provided). After a 5-week recovery period in one male, the prostate appeared normal and there was evidence of germinal epithelium regeneration with spermatogenesis.

In the second study (Cicmanec et al., 1991), four-month-old male and female beagle dogs (5 animals/sex/dose) were administered 0, 12.5, 39.5, or 72 mg/kg-day of dichloroacetate in gelatin capsules for 90 days. Testicular changes were reported in the males at all dose levels (except for control), including syncytial giant cell formation and degeneration of testicular germinal epithelium. Severity of the lesions increased in the mid- and high-dose animals (see also Table 5-5). Prostate glandular atrophy characterized by a significant reduction of glandular alveoli was also noted in mid-and high-dose groups. The testes of affected males did not show lesions upon gross necropsy. Absolute and relative testicular weights were unaffected by DCA treatment. A reproductive LOAEL of 12.5 mg/kg-day, the lowest dose tested, was established in this study. Data on the nonreproductive endpoints examined in this study are provided in Section 4.2.1.

4.4. OTHER STUDIES

4.4.1. Mechanistic Studies

A number of studies have evaluated the mechanism of action for DCA toxicity. Most, however, have concentrated on possible mechanisms for carcinogenicity rather than noncancer effects. Studies performed to elucidate the mechanism of toxicity of DCA have included *in vitro* and *in vivo* analyses with endpoints such as cell death, cell communication, response to growth factors, and the formation of tissue or DNA lesions.

In an effort to shed light on the cellular events preceding the development of malignant liver tumors in male $B6C3F_1$ mice, Carter et al. (2003) examined 1,355 slides from liver samples from 327 animals used by DeAngelo et al. (1999). Tissues collected from mice sacrificed throughout the DeAngelo et al. (1999) study were used to evaluate the effects of dose (0, 0.05 0.5, 1.0, 2.0 and 3.5 g/L DCA) and time (26, 52, 78 and 100 weeks) on liver lesions. Slides were processed for standard histological examination and were evaluated for the occurrence of altered hepatic foci (AHF), large foci of cellular alteration (LFCA, formerly called hyperplastic nodules), adenomas (AD) and carcinomas (CA). In order to minimize interhuman variability in the classification of tissue abnormalities, all of the slides were read by two observers who were blinded to treatment group and time-of-sacrifice.

In addition to the four main categories described above, lesions were subcharacterized into three groupings as follows: eosinophilic, dysplastic, and basophilic and/or clear cell. Eosinophilic cells showed increases in smooth endoplasmic reticulum and mitochondria. The dysplastic cells displayed atypical or enlarged nuclei. Tissue lesions from all four major categories and all three subcategories were identified in liver tissues from control and exposed animals. The basophilic cells had increased rough endoplasmic reticulum and/or ribosomes. The clear cells had accumulation of glycogen and/or lipids (steatosis).

When the histological examination of the tissues was completed, the data were arrayed by dose and time-to-sacrifice and reexamined to determine if there was a pattern of lesion progression with either dose or duration of exposure. The observed patterns of lesion frequency and their progression across the time- and dose-range gave rise to the hypothesis that there were three possible routes to the development of malignant tumors. In one case, eosinophilic cells seemed to progress from eosinophilic AHF to eosinophilic AD and CA. The basophilic cells and clear cells showed two patterns of progression. They either progressed from AHF to LFCA and then to CA or from LFCA to AD and then to CA. The dysplastic cells seemed to progress directly from AHF to CA. All three patterns of lesion progression were observed in the livers of mice treated with DCA and were significantly different from controls at some time or dose points. The majority of the cancers arose from the basophilic/clear cell progression.

The researchers also examined the relationship of necrosis, glycogen accumulation, cytomegaly, accumulation of lipid droplets, atypical nuclei, and enlarged nuclei to malignancies (Table 4-1). The strongest correlation was observed for cytomegaly. A correlation with glycogen accumulation and necrosis was observed for some doses but there was no consistent

dose-response pattern. The lack of dose-response at the high doses may be due to a decrease in the amount of liver tissue (non-involved liver) that had not been impacted by the tumors (AD or CA). While clear cells (lipid containing) were negatively correlated to the length of DCA exposure, this finding is consistent with the hypolipidemic effects of DCA.

Table 4-1. Biomarkers of tissue DCA exposure: incidence (%) of altered hepatic histology

Dose/Tissue Abnormality	Control	0.05 g/L	0.5 g/L	1.0 g/L	2.0 g/L	3.5 g/L
Cytomegaly	1.2	0	0	30.8	41.2	34.9
Glycogen	3.8	0	20	10.8	11.8	27.9
Steatosis	26.3	66.7	34.5	21.5	0	7
Necrosis	2.5	6.1	1.8	20	11.8	30.2
Atypical nuclei	22.5	33.3	32.7	55.4	58.8	46.5
Enlarged nuclei	41.2	39.4	54.5	55.4	45.1	41.9
CA	7.5	15.2	10.9	20	39.2	37.2

Source: Adapted from Carter et al. (2003).

Bruschi and Bull (1993) used hepatocyte suspensions from male $B6C3F_1$ mice and Sprague-Dawley rats to investigate the possible role of cytotoxic effects in DCA-induced hepatocarcinogenicity. Cytotoxicity was measured by the release of lactate dehydrogenase, trypan blue exclusion by the exposed cells,and depletion of intracellular reduced glutathione. No effects were seen in DCA-treated cells of either species using concentrations up to 5.0 mM and exposure times up to 240 minutes, suggesting little cytotoxicity from exposure to DCA as measured by the biomarkers employed.

Cellular changes that might indicate the potential mechanism of DCA-induced hepatotoxicity and carcinogenicity were studied in two parallel sets of experiments using the same strain of male mouse and an identical dosing regimen. In the first set of analyses, Carter et al. (1995) dosed male $B6C3F_1$ mice with 0, 0.5, or 5 g/L (0, 95, or 440 mg/kg-day, respectively) of DCA in drinking water for up to 30 days in two phases: Phase I was 5-15 days of treatment and Phase II was 20-30 days of treatment. Thymidine incorporation in hepatic DNA was measured by administering [^{3}H]-thymidine by a mini osmotic pump for 5 days prior to sacrifice. Groups of five animals were sacrificed at 5-day intervals. Significant, dose-related increases in absolute and relative (to total body weight) liver weights were seen at each 5-day interval. These

trends increased with the length of exposure. Hepatocytes from Phase I animals in the high-dose group exhibited reduced thymidine incorporation (labeling index) and inhibition of mitosis. In Phase II, a decrease in the labeling index was observed among the low- and high-dose groups. Differences from the control group were significant at 20 and 25 days, but not at 30 days. Both treatment groups had enlarged nuclei, which may suggest polyploidy. The hepatocytes also exhibited glycogen accumulation, suggesting alterations in cellular metabolism. The authors concluded that DCA exposure initially inhibits rather than stimulates cell proliferation (hyperplasia), and that the increased liver weight is due to hepatocyte enlargement rather than regenerative hyperplasia following cell death.

A second segment to the Carter et al. (1995) project examined the role of apoptosis (programmed cell death) suppression as a contributing factor to DCA-induced hepatocarcinogenicity. The results were published as Snyder et al. (1995). Apoptotic cells were visualized by *in situ* nick-end labeling of DNA from the livers of animals sacrificed at 5-day intervals. Regression analysis revealed a significant trend toward decreased apoptosis as the dose and length of exposure increased. The lowest dose, 0.5 g/L, was shown to significantly ($p<0.05$) decrease apoptosis at the earliest time point (5 days) and also at days 15, 25, and 30. For the high-dose group, apoptosis was significantly depressed as compared to controls for all time points except the 20-day point. The authors suggested that DCA may suppress the apoptotic mechanism by which initiated tumor cells would otherwise be removed.

Benane et al. (1996) examined the effects of 1-, 4-, 6-, 24-, 48-, and 168-hour exposures to DCA (0, 5, 10, or 50 mM) on gap junction intercellular communication in Clone 9 cell cultures (normal rat hepatocytes). No differences in intercellular communication were seen between the 5 mM groups and controls, as measured by a dye transfer protocol, but there was a difference between all 50 mM groups and controls. The shortest exposure time and lowest exposure concentration which significantly reduced dye transfer was for the 6-hour, 10 mM group. A 41 mM DCA concentration produced a 50% reduction in dye transfer over a 24-hour period. The significance of the disruption in intercellular communication has not been elucidated, but DCA's ability to disrupt communication was much weaker ($\geq$5.8-fold) than other chlorinated compounds tested, including: perchloroethylene, trichloroacetic acid, trichloroethanol, and chloral hydrate.

Tsai and DeAngelo (1996) examined the effects of DCA administered to male $B6C3F_1$ mice on the subsequent responsiveness to growth factors of isolated hepatocytes in culture.

Mice were administered drinking water with 0 or 3.5 g/L DCA for up to 90 days. Incorporation of [^{3}H]-thymidine in the presence of epidermal, hepatocyte, or acidic fibroblast growth factors was then measured in the isolated hepatocytes, with or without the mito-inhibitory transforming growth factor β_1. Inhibition of basal DNA synthesis was noted in cells isolated from animals exposed to DCA for 30, 60, or 90 days. However, cells from DCA-treated mice that were treated in culture with growth factors exhibited enhanced DNA synthesis similar to that seen in cultured cells from control mice. The authors suggested that the early depression of cell proliferation seen in other studies of DCA-induced tumorigenesis is due to some mechanism other than an impaired ability to respond to growth factors.

As knowledge of the complex sequence of cytosolic and nuclear events that influence neoplasia increases, it is important to consider data on genetically-linked events including changes in the genetic messages (proto-oncogenes) for transcription factors and signal transduction proteins. The data base for DCA includes the results of several studies that examined the *ras* signal transduction genes, plus the *jun*, *fos* and *myc* transcription factors.

Anna et al. (1994) exposed male B6C3F$_1$ mice to drinking water containing 0 (50 animals) or 5 g/L DCA (110 animals, about 900 mg/kg-day), 5 days/week for 76 weeks. Mice treated with DCA had an increased incidence of both hepatic adenomas (93% of treated mice had at least one adenoma vs. 8% positive for control animals), as well as hepatocarcinomas (74% of the treated mice had at least one carcinoma vs. 8% for control animals). There were no significant differences in H-*ras* codon 61 mutation frequency among DCA-induced and spontaneous hepatocellular tumors. However, significant changes were seen in the mutation spectra of H-*ras* codon 61 in the DCA-treated mice as compared to the control animals. In the spontaneous tumors from the controls (study controls plus historical controls) the CAA of codon 61 became AAA in 59 % of the tumors, CGA in 28% and CTA in 14%. In the DCA-treated mice, the H-*ras* codon 61 changes were 28% AAA, 35% CGA and 38% CTA. The authors suggest that these differences were due to nonspecific secondary DNA damage by DCA. The authors further suggest that DCA exposure, while not necessarily causing mutations in the H-*ras* gene, may nevertheless provide a selective growth advantage to mutations that arise spontaneously**.** *Ras* proteins are GTPases that are involved in the activation of a series of protein kinases that control cell growth and differentiation. *Ras* is activated by binding of a ligand to a cell surface receptor.

The findings of Anna et al. (1994) are partially supported by those of Velazquez (1995) in which DNA was examined from normal liver and tumor tissues obtained from male $B6C3F_1$ mice that were administered 0.5 g/L (90 mg/kg-day) DCA in drinking water for 2 years. Sequences of the H-*ras* gene were amplified using PCR (polymerase chain reaction); it was observed that H-*ras* codon 61 mutations were present in three out of five (60%) of the DCA-induced tumors. In this case, the spectrum of mutations associated with DCA was the same as that of spontaneous tumors from untreated animals. The significance of this observation is limited by the fact that there were only three tumors with codon 61 mutations.

In another study, male $B6C3F_1$ mice were administered 1.0 or 3.5 g/L (180 or 630 mg/kg-day) DCA in drinking water for 104 weeks, and then sacrificed (Ferreira-Gonzalez et al., 1995). The incidence of liver carcinomas was 19% in the untreated mice, 70.6% in the 180 mg/kg-day group, and 100% in the 630 mg/kg-day group. DNA samples were examined from 32 spontaneous liver tumors from the control group, 13 tumors from the 180 mg/kg-day group, and 33 tumors from the 630 mg/kg-day group. The DNA was analyzed for K- and H-*ras* proto-oncogene mutations in the DCA-induced and spontaneous tumors. Point mutations in exons 1, 2, and 3 of the K- and H-*ras* genes were quantified by single-stranded conformation polymorphism. Similar frequencies of H-*ras* proto-oncogene exon 2 mutation were found in all three groups (58% in spontaneous tumors, 46% in 180 mg/kg-day group, and 50% in the 630 mg/kg-day group). Mutation frequencies in other exons were minimal.

Comparative sequence analysis of exon 2 mutations from spontaneous and DCA-induced tumors revealed a substantial shift in the spectrum of base changes in codon 61. Sequence analysis of spontaneous tumors revealed changes in codon 61 from CAA to AAA in 80% and CAA to CGA in 20% of the examined tumors (Table 4-2). No CAA to CTA conversion was observed in spontaneous tumors. In contrast, the frequency of CAA to AAA conversion was 16% and 21% at DCA doses of 180 and 630 mg/kg-day, respectively. CAA to CGA conversion was noted in 50% of the tumors from mice treated with either 180 or 630 mg/kg-day, and CAA to CTA conversion was observed in 34% and 29% of the two dosage groups, respectively. Thus, although DCA-induced and spontaneous tumors involved similar levels of H-*ras* mutation, the mechanisms of tumor induction may be different. Differences in codon 61 mutation spectra between spontaneous and DCA-induced tumors in this study are similar to those reported in the Anna et al. (1994) study, where there was also a lower number of CAA to AAA conversions and a higher number of CAA to CTA conversions in the DCA-induced tumors as opposed to the spontaneous tumors.

Table 4-2. Frequency of spontaneous and DCA-induced mutations of codon 61 in exon 2 of the H-*ras* oncogene mutations in B6C3F$_1$ mice

Dose (mg/kg-day)	Mutation Frequency (%)		
	AAA	CGA	CTA
Untreated (spontaneous mutations)	80	20	--
180	16	50	34
630	21	50	29

Source: Adapted from Ferreira-Gonzalez et al. (1995).

Schroeder et al. (1997) examined DCA-induced tumors in female B6C3F$_1$ mice for H-*ras* codon 61 mutations. There was a H-*ras* mutation in only one of 22 tumors. However, this one mutation was consistent with the observations of other researchers in that it involved a CAA to CTA conversion.

Stauber et al. (1998) demonstrated that DCA increases cell proliferation of c-*Jun* positive hepatocytes *in vitro*. As mentioned previously, c-*Jun* is a nuclear transcription factor that is associated with apoptosis and cell transformation. Its expression is linked to the H-*ras* signal transduction cascade (Johnson et al., 1996). The investigators treated isolated hepatocytes from neonatal mice with DCA and plated the cells to allow them to form colonies. Exposure of the cells to 0.5 mM DCA significantly increased colony formation (no cytotoxicity) over controls. Interestingly, the colonies that were induced by DCA were c-*Jun* positive. This is noteworthy because this is the same phenotype observed in DCA-induced liver tumors in whole mice exposed to DCA (Stauber and Bull, 1997). When mice were pretreated for 2 weeks with DCA in their drinking water prior to preparation of hepatocytes, DCA again induced c-*Jun* positive colony formation, but only required 0.02 mM DCA for the same degree of induction.

While Pereira et al. (2001) investigated the effect of DCA treatment on proto-oncogene gene expression in the liver, the study considered the effect of DCA treatment on the hypomethylation and expression of the c-*myc* gene and the promotion of liver and kidney tumors. The c-*Myc* gene is a nuclear protein that is involved in transcriptional response and proliferation of liver cells. Hypomethylation of the c-*myc* gene seems to enhance its expression and thus cell division.

In the first of two experiments by Pereira et al. (2001), 7- to 8-week-old female $B6C3F_1$ mice were administered 400, 800, or 1600 mg/L chloroform in drinking water for 17 days. On the last 5 days of treatment, the mice were also administered 500 mg/kg-day of DCA via gavage. Methylation of the c-*myc* gene was determined by enzymatic DNA hydrolysis using a HpaII restriction endonuclease to digest unmethylated CCGG sites combined with Southern blot analysis. Gene expression was evaluated using Northern blot analysis for c-*myc* mRNA. Dichloroacetic acid decreased c-*myc* methylation and increased expression of the gene more than chloroform. Doses ≥800 mg/kg-day chloroform, coadministered with DCA, significantly reduced the ability of DCA to increase gene expression.

In the second experiment, five-week-old male and female $B6C3F_1$ mice were administered 3.2 g/L DCA in drinking water, either alone, or in conjunction with 800 or 1600 mg/L chloroform (Pereira et al., 2001). Prior to DCA exposure, the mice had been initiated with a single (300 mg/kg) intraperitoneal dose of MNU at 15 days of age. The mice were sacrificed at 36 weeks of age. Greater numbers of hepatic foci were observed in DCA-treated animals (females more than in males). The tumor response was greater in males than in females. Chloroform in conjunction with DCA at both doses drastically reduced the adenomas and adenocarcinomas. One interesting effect of combining DCA exposure with chloroform in the MNU-treated mice was the occurrence of kidney tumors. While treatment alone produced few tumors in the kidney, coexposure with chloroform increased the tumor multiplicity.

Thai et al. (2001) investigated changes in early gene expression in mice liver following DCA exposure. Four-week-old mice were administered 2 g/L DCA in drinking water for 4 weeks. Differential display of mRNA levels revealed that 381 genes showed differences in intensity of the display between the exposed mice and the controls. Upon further refinement of the data, six genes were identified that were expressed differently in control and exposed mice (one gene induced, the other five suppressed). Four genes were identified: stearoyl-CoA desaturase was induced, while alpha-1 protease inhibitor, cytochrome b_5, and carboxylesterase were suppressed. All but alpha-1 protease inhibitor are endoplasmic reticular enzymes involved in fatty acid metabolism. Four of the six genes were found to be similar in hepatocellular carcinomas (from additional mice treated with 3.5 g/L DCA for 93 weeks) and in the livers of mice treated with DCA for 4 weeks. The identified genes that were similar in the tumors and the DCA-treated mice were those for alpha-1 protease inhibitor, cytochrome b_5, carboxylesterase, and an unnamed gene. The expression of stearoyl-CoA desaturase and one other identified gene were the same in the control mice and the tumors. The significance of these findings is

unknown, relative to the carcinogenic properties of DCA. Changes in the expression of some of the genes may merely reflect metabolic perturbations induced by DCA rather than cancer-linked events.

A second study by the same researchers (Thai et al., 2003) utilized the same mouse liver tissue samples and gene microarrays. The first array was the Atlas Mouse Cancer 1.2 Array, which contains 1,176 unique complementary cDNA fragments from genes known to be implicated in cancer development (Clontech, 2000). The second was an array of 140 genes representing mouse stress/toxicity response elements. There were approximately 50 genes that were common to the two microarrays. In the mouse stress/toxicity array there were 13 genes that were differentially expressed: five showed increased expression and 8 decreased expression. From the mouse 1.2 cDNA array, 11 genes were differentially expressed; the expression of two was increased while expression of nine was decreased. Thai et al. (2003) considered the results of their 2001 and 2003 analyses and concluded that the affected genes were related to three cell-response groupings: tissue remodeling and/or angiogenesis, xenobiotic metabolism, and damage response. Most of the genes in each of these groupings were suppressed. The authors hypothesized that the suppressed gene expression in the tissue remodeling and angiogenesis group plus the tissue repair grouping facilitated tumor growth. The GSTZ gene and other genes involved in glycogen and lipid metabolism were not present in the microarrays that Thai et al. (2003) employed. The PPARα gene was present but not activated in the microarray.

On the other hand, the finding that DCA induced peroxisomal enzymes in some studies, suggested that the PPARα gene can be activated. Since peroxisomes generate hydrogen peroxide through some of their metabolic reactions, they are often associated with oxidative change to cellular DNA. Austin et al. (1996) investigated the potential for DCA to increase intercellular lipid peroxidation and the oxidation of DNA. Male B6C3F_1 mice were treated with a single oral dose of DCA (0, 30, 100, or 300 mg/kg). Nuclear DNA was extracted at various times in order to assess increases in relative guanosine hydroxylation. A significant increase was seen in the 300 mg/kg group from 4 to 6 hours postdosing, but returned to near control levels at 8 hours postdosing. The authors suggest that DNA hydroxylation indicates oxidative stress induced by DCA in mouse livers. The level of hydroxylation appeared to be related to the ability to induce thiobarbituric acid-relative substances (TBARS), which is an indicator of lipid peroxidation. Significant increases in lipid peroxidation have also been shown in cultured primary rat and mouse hepatocytes following exposure to DCA concentrations as low as 0.5 mM (mouse) and 1.0 mM (rat; Everhart et al., 1998).

4.4.2. Genotoxicity Studies

Observations on DCA

There have been multiple studies investigating the hypothesis that DCA is a genotoxic agent. Results from *in vitro* studies are summarized in Table 4-3. The majority of these studies indicate that DCA is only genotoxic at high doses or after long durations. Most of the *in vitro* tests are negative or equivocal, either in the presence or absence of metabolic activation.

While one report indicated that DCA may increase prophage λ induction in *E. coli* (DeMarini et al., 1994), this finding has not been confirmed by other laboratories and required DCA concentrations in the mM range to achieve significance. In the Ames assay, DCA has been evaluated using strains TA98, 100, 1535, 1537, 1538, 1950, 2322, and TS24 in the presence and absence of S9 activation (Fox et al., 1996; Herbert et al., 1980; Waskell, 1978). The only clear positive results were reported by DeMarini et al. (1994) using DCA in the vapor phase and strain TA100. However, the results have not been replicated in other reversion assays and it is possible that the form of DCA affected the study results. The increased revertants may be the result of low pH resulting from the use of the free acid in the vapor phase or differences in the membrane transport of the non-ionized acid.

Herbert et al. (1980) reported an equivocal increase in revertants in strains TA98 and TA1538 when exposed to 1-10 μg/plate DCA (salt); all other strains gave negative results. The revertant numbers were similar in both the presence and absence of metabolic activation, ranging from ~64 to 102 revertants/plate (compared to negative control values of 59-61 revertants/plate). The response in TA98 was considered by the study authors to be evidence of a weak mutagenic effect because the response in TA1538 was not unequivocally dose-related. The results should not be considered strong evidence for the mutagenic capacity of DCA. Only the results in TA98 were presented in the published paper and they show a slight increase above the spontaneous reversion rate. The increase (1.4- to 1.7-fold) did not reach the limit (2- to 3-fold) that most laboratories would typically require for the compound to be identified as mutagenic. The dose-response trend reached statistical significance, however, in both the absence and presence of S9. Nevertheless, the authors could not exclude the possibility that the mutagenicity observed was the result of a contaminant in the DCA.

Data from genotoxicity assays using mammalian cells in culture provided negative results. Harrington-Brock et al. (1998) reported that DCA induces mutations at the thymidine kinase locus, as well as gross chromosomal aberrations in L5178Y mouse lymphoma cells *in vitro*, but the concentrations required to induce these effects were in the mM range. Additionally, DCA did not induce micronuclei formation in the cells. The authors compared the dose-response curve of DCA mutagenicity to that of ethylmethane sulfonate, noting that the mutagenic potency was similar to, but less than, that of the classic mutagen. Recognizing that their data provides evidence for the mutagenic capacity of DCA, the authors noted that the compound is unlikely to be a mutagen at concentrations found in finished drinking water (Harrington-Brock et al., 1998). The results of studies of DNA strand breaks in several cell lines (Chang et al., 1992), Chinese hamster ovary cell chromosomal aberrations (Fox et al., 1996) and DNA repair (Waskell, 1978) were negative in the absence of S9 activation. The chromosomal aberration assay was the only one conducted in the presence of the microsomal S9 factor and those results were also negative.

Table 4-3. Summary of *in vitro* genotoxicity tests

Assay	Result		Concentration	Reference
	Without S9	With S9		
λ Prophage induction in *Escherichia coli* WP2	+	+	2,500 µg/mL	DeMarini et al., 1994
Bacterial reverse mutation assay				
TS 24	-	-	31,000 µg/mL	Waskell, 1978
TA 2322	-	-	31,000 µg/mL	Waskell, 1978
TA 1950	-	-	31,000 µg/mL	Waskell, 1978
TA 100	-	-	5,000 µg/plate	Fox et al., 1996
	-	-	1-10 µg/plate	Herbert et al., 1980
	+	+	1 µg/mL	DeMarini et al., 1994
	-	-	NA	Matsuda et al., 1991
	+	+	100-1,500 µg/mL (-S9); 1,500-7,500 µg/mL (+S9)	Giller et al., 1997
TA 1535	-	-	1-10 µg/plate	Herbert et al., 1980
	-	-	5,000 µg/plate	Fox et al., 1996
TA 1537	-	-	1-10 µg/plate	Herbert et al., 1980
	-	-	5,000 µg/plate	Fox et al., 1996
TA 1538	+(a)	+(a)	1-10 µg/plate	Herbert et al., 1980
	-	-	5,000 µg/plate	Fox et al., 1996
TA 98	+	+	1-10 µg/plate	Herbert et al., 1980
	-	-	5,000 µg/plate	Fox et al., 1996
E. coli WP2uvrA	-	-	5,000 µg/ plate	Fox et al., 1996
DNA strand breaks				
• mouse hepatocytes	+(b)		2,580 µg/mL	Chang et al., 1992
• rat hepatocytes	-		1,290 µg/mL	Chang et al., 1992
• human CCRF-CEM cells	-		1,290 µg/mL	Chang et al., 1992

Assay	Result		Concentration	Reference
	Without S9	With S9		
L5178Y/TK$^{+/-}$ mouse lymphoma mutation assay	- +(c) -(d)	NA NA	5,000 µg/mL 600-800 µg/mL	Fox et al., 1996 Harrington-Brock et al., 1998
Chromosome aberration assay (Chinese hamster ovary cells)	-	-	5,000 µg/mL	Fox et al., 1996
Newt micronucleus test	NA	-(e)	20, 40, 80 µg/mL	Giller et al., 1997
DNA repair Repair deficient strains TA1535 (umu operon) E.coli PQ37	 - - +	 NA + -	 31 mg/plate 58.5 µg/mL 500 µg/mL	Waskell, 1978 Ono et al., 1991 Giller et al., 1997

NA=not applicable/not available
(a) The results in TA1538 were positive but did not "exhibit an unequivocal dose-response relationship" (Herbert et al., 1980).
(b) Small increase in strand breakage (7%) seen after 4-hour exposure, but not at 1 hour; response deemed negligible.
(c) Mutations/chromosome aberrations.
(d) Micronuclei induction.
(e)Test is performed using stage 53 newt larvae in the absence of exogenous S9; any metabolic activation is from the test animal.

Results from *in vivo* studies are shown in Table 4-4. In this case, results are mixed with no consistent pattern of positive or negative results for mouse micronucleus assay, DNA strand breaks in mouse and rat cells, or DNA adduct formation. In particular, DCA has been investigated *in vivo* for its ability to induce single-strand breaks in DNA. Chang et al. (1992) exposed B6C3F$_1$ mice to drinking water containing 0.05, 0.5, or 5.0 g/L DCA for 7 and 14 days while F344 rats were exposed to drinking water containing 0.05, 0.5 or 2 g/L DCA for 30 weeks. Analysis of damaged DNA was conducted in hepatocytes as well as in epithelial cells taken from the spleen, stomach and duodenum. Consistent with their *in vitro* results, the authors reported no evidence of increased DNA strand breakage at any dose tested in mice or rats. While the authors reported a 7% increase in strand breaks in mice dosed *in vivo*, they considered this result to be insignificant.

The findings by Chang et al. (1992) are in direct opposition with earlier work published by Nelson et al. (1989) and Nelson and Bull (1988). In these two studies, DCA exposure significantly increased DNA single-strand breaks in the livers of mice and rats. It is interesting

to note that the reported DNA damage occurred at an oral dose of 10-13 mg/kg-day, almost 2 orders of magnitude lower than the doses used in the Chang et al. (1992) study. The basis of the differences in results between laboratories is not clear but may be the result of differences in methodology. The results of Nelson et al. (1989) and Nelson and Bull (1988) may reflect the rapid repair of hydroxylated guanines (Austin et al., 1996), which require the formation of single-strand breaks. Fuscoe et al. (1996) reported no significant increase in DNA migration (evidence of DNA strand breaks) in mice exposed *in vivo* up to the highest concentration of 3.5 g/L. At the highest dose, however, there was a reduction in migration rates that was interpreted to be evidence of DNA cross-linking. This result is in contrast with the negative findings of other assays, which can also measure DNA cross-linking (Chang et al., 1992; Fox et al., 1996).

Table 4-4. Summary of *in vivo* genotoxicity tests

Assay	Result	Concentration or Dose	Duration	Reference
Micronuclei assay (mouse)	+ - -	3.5 g/L 3.5 g/L 1,000 mg/kg	9 days 28 days 3 days	Fuscoe et al., 1996 Fuscoe et al., 1996 Fox et al., 1996
DNA Strand Breaks				
Mouse leukocytes	-	3.5 g/L	28 days	Fuscoe et al., 1996
Mouse hepatocytes	+ + -	13 mg/kg 10 mg/kg 0.05, 0.5, 5 g/L (1,290 mg/kg)	1 dose 1 dose 7 & 14 days	Nelson and Bull, 1988 Nelson et al., 1989 Chang et al., 1992
Mouse splenocytes	-	5 g/L (1,290 mg/kg)	14 days	Chang et al., 1992
Mouse epithelial cells (a)	-	5 g/L (1,290 mg/kg)	14 days	Chang et al., 1992
Fischer rat hepatocytes	-	0.05, 0.5, 2 g/L	30 weeks	Chang et al., 1992
Sprague-Dawley rat hepatocytes	+	30 mg/kg	1 dose	Nelson and Bull, 1988
8-OH DNA adducts	+ -	oral 300 mg/kg up to 2.0 g/L	1 dose (gavage) 3 & 10 weeks	Austin et al., 1996 Parrish et al., 1996
Lac I operon transgenic mutations	+	1 and 3.5 g/L	60 weeks	Leavitt et al., 1997

(a) Epithelial cells from the stomach and duodenum.

In an *in vivo* micronucleus assay, Fox et al. (1996) exposed Sprague-Dawley rats intravenously to 275, 550, and 1,100 mg/kg DCA, and did not detect an effect. Fuscoe et al. (1996) evaluated micronuclei induction in poly- and normochromatic erythrocytes (PCEs and

NCEs, respectively) in male B6C3F$_1$ mice following *in vivo* exposure to drinking water containing DCA at approximate doses of 95, 190, 380, or 665 mg/kg-day for up to 28 days in one experiment and 31 weeks in another experiment. In the first study, an increase in micronucleated PCEs was noted in the high-dose group, but only at day 9. This was apparently a transient effect and by day 28, the increased incidence of micronuclei was no longer evident. There was also no increase in micronucleated NCEs. It should be noted, however, that the control frequency was twice as high in the 28-day study as in the 9-day study, which might have affected the ability of the assay to detect slight increases in micronuclei.

Fuscoe et al. (1996) also measured the effect of tocopherol (vitamin E) administration on the induction of micronuclei at the high dose (665 mg/kg-day) at both 9 and 28 days (to determine if increased intracellular oxygen radicals were causing the DNA damage). Vitamin E treatment had no effect on micronuclei formation. Interestingly, doses of 665 mg/kg-day DCA plus vitamin E significantly increased micronuclei at 9 and 28 days, when compared to the vitamin E controls.

In the second experiment, mice were administered 665 mg/kg-day DCA for 10, 26, or 31 weeks (with water administered alone following the exposure period up to sacrifice at 31 weeks). At each time point, slight but significant increases in NCEs were observed while micronucleated PCEs slightly increased in a dose-dependent manner, but did not reach statistical significance. The response was greater for PCEs than for NCEs. Data reflected the much higher control micronuclei frequency for PCEs than for NCEs (Fuscoe et al., 1996).

Austin et al. (1996) reported increases in the DNA adduct 8-hydroxy-2-deoxyguanosine in DCA-treated mice. The increase was noted in animals at all doses tested (single doses of 30, 100 or 300 mg/kg DCA), but was statistically significant only in the high-dose group (300 mg/kg) and only at 4 and 6 hours postdosing. This finding was interpreted to indicate the potential for DCA to oxidatively damage hepatic DNA. In contrast, Parrish et al. (1996), treated B6C3F$_1$ male mice for 10 weeks with 540 mg/kg-day DCA and saw no evidence of increased 8-hydroxy-2-deoxyguanosine.

Leavitt et al. (1997) exposed transgenic mice (Big Blue) to 1 or 3.5 g/L DCA (approximate doses of 190 or 665 mg/kg-day) in their drinking water for 60 weeks. The concentrations were comparable to those used in chronic bioassays. At interim time points (4 and 10 weeks), neither concentration of DCA induced an increased frequency of mutations in the Lac

I loci. However, at 60 weeks, both concentrations of DCA induced a significantly elevated mutational frequency at this loci. This time-response pattern suggests that the mutational events might be secondary to toxicological changes in the liver rather than a direct genotoxic effect, since a direct effect would be expected to be time-independent. The results indicate that a large cumulative dose (due to the 60-week exposure period) is necessary to increase mutations in this *in vivo* system. A second complicating issue regarding this study is the clonal expansion of preneoplastic and neoplastic cells within the tissue, which may account for the apparent increase in mutation rate at 60 weeks (WHO, 2000). Although, the investigators accounted for this potential confounder by analyzing the type of mutation (i.e., base substitutions) and subtracting duplicate identical mutations recovered in the same animal, the proportion of mutation types recovered from control and treated mice were still statistically different after the adjustment. The study authors, however, did not provide any justification for this correction.

Observations on DCA Metabolites

In contrast to the findings reported for DCA, glyoxylate has been shown to be mutagenic in four independent studies (Marnett et al., 1985; Sasaki and Endo, 1978; Yamaguchi and Nakagawa, 1993; Sayato et al., 1987). However, the concentrations of glyoxylate required to produce positive results are very high (in the mM range) and it is not known whether these can be reasonably achieved *in vivo* from the metabolism of DCA. Consequently, it is uncertain whether the results are likely to be relevant to the issue of DCA genotoxicity. Haworth et al. (1983) reported that oxalate was not mutagenic in *Salmonella*.

Summary

The genotoxicity/mutagenicity of DCA has been investigated in a number of studies. The preponderance of *in vitro* studies are negative, with only a few equivocal or positive results. Studies *in vivo* are mixed, with internally inconsistent results between studies and between endpoints. The difference in results does not appear to be clearly related to differences in exposure levels. While Leavitt et al. (1997) found an increased frequency of mutations in the Lac I loci after exposure for 60 weeks, this was not observed at interim time points (4 and 10 weeks). The findings suggest that duration of exposure may be an important variable. The importance of these findings and the potential relevance to the issue of DCA carcinogenesis is further discussed in Section 4.6.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

The noncancer effects of DCA can be grouped into one of four major categories: metabolic alterations, hepatic toxicity, reproductive/developmental toxicity, and neurotoxicity. Important observations on each of these effect categories are briefly summarized below.

4.5.1. Metabolic Alterations

Multiple independent studies demonstrate that DCA has the ability to alter normal carbohydrate metabolism. Dichloroacetic acid treatment results in a significant reduction in plasma levels of glucose, pyruvate, and lactate. This finding has been consistently reported in DCA-treated rats, dogs and humans (Ribes et al., 1979; Katz et al., 1981; Evans and Stacpoole, 1982; Davis, 1986, 1990; Stacpoole et al., 1978). The primary mechanism of action associated with the decrease in blood glucose and lactic acid appears to be enhancement of pyruvate dehydrogenase activity (Crabb et al., 1981; Stacpoole, 1989). This is the enzyme that decarboxylates pyruvate and provides acetyl-CoA for the citric acid cycle. Activation of pyruvate dehydrogenase occurs indirectly by DCA inhibition of the protein kinase that maintains it in its inactive form (Whitehouse et al., 1974; Stacpoole, 1989). By stimulating the pyruvate dehydrogenase, DCA accelerates pyruvate, lactate, and alanine oxidation, and results in plasma level decreases of these metabolites. As lactate is oxidized first to pyruvate and then to acetyl-CoA by pyruvate dehydrogenase, there is a corresponding decrease in the hydrogen ions that exist in a 1:1 stoichiometry with lactate, and the subsequent generation of bicarbarbonate ions. This serves as the basis for using DCA in the treatment of severe cases of lactic acidosis (Stacpoole et al., 1998a). Similarly, by the removal of lactate and alanine, two major gluconeogenic substrates, DCA inhibits hepatic glucose output and induces the resulting decrease in circulating glucose levels (Stacpoole et al., 1998a).

A potential confounder in the interpretation of some studies of the effect of DCA on metabolism is an exposure-related decrease in water and food consumption, especially in the high-dose groups (Bhat et al., 1991; Katz et al., 1981; Davis, 1986; Mather et al., 1990; Yount et al., 1982). A drop in water and food consumption could contribute to the reported decreases in body weight observed in some high-dose group animals and could potentially impact glucose metabolism as well. The relationship between nutritional status and aerobic glycolysis is

complex, but glucose metabolism could clearly be modified by a significant decrease in the caloric intake of treated animals. However, changes in plasma levels of glucose or lactic acid have been seen in DCA-treated humans with no associated weight loss. Additionally, metabolic changes have been consistently observed in DCA-treated animals at doses below those resulting in body weight changes. Therefore, metabolic effects are not artifacts of altered food or water intake.

Dichloroacetic acid exposure also results in a decrease in plasma cholesterol levels. This has been observed in experimental animals and humans, and was even briefly exploited therapeutically in the treatment of a few individuals with hypercholesterolemia (Moore et al., 1979; Stacpoole et al., 1978). Dichloroacetic acid has been shown to be a noncompetitive inhibitor of the rate-limiting microsomal enzyme in cholesterol biosynthesis, hydroxymethylglutaryl (HMG) CoA reductase (Stacpoole, 1989). It also inhibits hepatic synthesis of triglycerides by an unknown mechanism (Stacpoole and Greene, 1992). The net effect of these inhibitory activities is a decrease in serum lipids and lipoproteins *in vivo* following DCA dosing. All DCA-induced metabolic alterations appear to be transient, with full recovery to basal/control levels observed following cessation of DCA administration.

Cornett et al. (1999) demonstrated that DCA can significantly alter tyrosine metabolism as a consequence of its inhibitory effect on GSTZ. Inhibition of tyrosine metabolism can result in increased levels of reactive tyrosine metabolites such as maleylacetoacetate and maleylacetone, metabolites that may adversely affect the heart, liver and nerves, targets of DCA toxicity. In humans, hereditary tyrosinemia II (a disease involving a deficit in tyrosine metabolism) is often associated with the development of polyneuropathy, and/or hypertrophic cardiac myopathy in young patients (Tanguay et al., 1996; LaBerge et al., 1986).

4.5.2. Hepatic Toxicity

Another consistent finding in DCA ingestion studies is a dose-related increase in liver size (DeAngelo et al., 1999; Sanchez and Bull, 1990; Yount et al., 1982; Mather et al., 1990; Smith et al., 1992), generally accompanied (or caused) by an increase in glycogen deposition in the liver (Kato-Weinstein et al., 1998; Bhat et al., 1991). The enzymatic basis for increased hepatic glycogen accumulation remains unclear, although it has been shown that DCA treatment does not alter glycogen synthetase or the amount of active hepatic phosphorylase (Kato-

Weinstein et al., 1998). The increase in liver size and glycogen accumulation resemble changes occurring in glycogen storage disease, suggesting that failure of glycogenolysis, through either glycogen phosphorylase or a debranching enzyme, may play a role in the observed accumulation. The dose-response for glycogen deposition in the liver is in the same range that is required for inducing hepatocarcinogenesis (Bull, 2000).

The glycogen accumulation and hepatomegaly observed in DCA-treated rats are similar to changes observed in humans with glycogen storage disease VI. This human genetic disorder is believed to be the result of a deficiency in liver, rather than muscle, glycogen phosphorylase b kinase. This kinase is responsible for the conversion of inactive glycogen phosphorylase b to active glycogen phosphorylase a. The symptoms of glycogen storage disease VI include accumulation of liver glycogen, liver enlargement and a tendency for development of liver adenomas and carcinomas (Hers et al., 1989). The disorder is also marked by increased levels of plasma cholesterol and triglycerides in some subjects (Hers et al., 1989). The increase in plasma lipids is different from the typical decrease in lipids observed following DCA exposure. It can be noted that some, but not all, of the cancers observed by DeAngelo et al. (1999) in mice appear to have originated from the clear cells involved in glycogen storage (Carter at al., 2003).

Liver toxicity, as evidenced by increases in serum levels of liver enzymes, has been seen in DCA-treated mice, rats, dogs and humans (DeAngelo et al., 1991, 1999; Mather et al., 1990; Cicmanec et al., 1991; Stacpoole et al., 1998a; Katz et al., 1981). Frank hepatic cytotoxicity in the form of necrosis has been consistently reported in DCA-treated mice, with exposure levels of 0.5 g/L (~77 mg/kg-day) associated with necrosis of scattered individual hepatocytes, and exposures of 1-5 g/L (~150 to ~1000 mg/kg-day) resulting in larger areas of coagulative necrosis (DeAngelo et al., 1991; Bull et al., 1990; Daniel et al., 1992; Sanchez and Bull, 1990; ILSI, 1997). Interestingly, frank liver necrosis has not been seen in rats, even at the highest concentration used (5 g/L) (DeAngelo et al., 1996), nor has it been reported in dogs or humans. The reason for the preferential severity of the hepatotoxic response in mice is not known. Sanchez and Bull (1990) suggested that liver necrosis observed in DCA-treated mice was not the result of DCA-induced hepatocytotoxicity per se, but occurred in infarcted areas caused by extensive liver hypertrophy.

Another hypothesis is that liver necrosis is secondary to lipid peroxidation. This is supported by evidence of oxidative damage to hepatic DNA in DCA-treated mice (Austin et al., 1996). Though direct evidence of lipid peroxidation in the mouse is limited, this observation suggests the potential for DCA to oxidatively damage the liver. Evidence of lipoperoxidation

has also been reported in Fischer 344 rats treated with doses of 300-1000 mg/kg-day (Larson and Bull, 1992). However, this finding was not confirmed by Mather et al. (1990) who reported no evidence of lipoperoxidation in Sprague-Dawley rats treated with doses of 50-250 mg/kg-day. Nevertheless, the fact that necrosis has been observed at concentrations that have not been shown to induce lipid peroxidation (~77-159 mg/kg-day) argues against this being a significant mechanism of cell death.

4.5.3. Reproductive/Developmental Toxicity

There is an extensive and consistent data base demonstrating the reproductive toxicity of DCA in males and females (Katz et al., 1981; Yount et al., 1982; Bhat et al., 1991; Cicmanec et al., 1991; Toth et al., 1992; DeAngelo et al., 1996; Linder et al., 1997; Smith et al., 1992; Epstein et al., 1992). Section 4.3 presents further details of these studies. In male rats, DCA may cause decreases in testicular weight at 1,100 mg/kg and viable sperm production at 62.5 mg/kg-day. While testicular degeneration was observed in rats at 500 mg/kg-day and dogs at 12.5 mg/kg-day, it has not been reported in exposed humans. However, testicular effects in humans have not been specifically examined, because they cannot be readily assessed by noninvasive techniques. In female rats, DCA exposure to dose levels of 140 mg/kg-day during gestation can lead to impaired fetal maturation and result in soft tissue anomalies (primarily of cardiac origin) in the offspring (Smith et al., 1992; Epstein et al., 1992).

To date, no specific cellular or molecular mechanism of action has been proposed to explain the testicular or developmental toxicity associated with DCA administration. Dichloroacetic acid can freely and rapidly cross the placenta (Smith et al., 1992); however, during early organogenesis, the embryo relies almost exclusively on glycolysis for energy, a process stimulated by DCA (Smith et al., 1992). It is not known if the DCA-mediated effect on glycolysis may be relevant to the mechanism of action of developmental toxicity. Although the mechanism by which DCA targets the embryonic heart is not clear, there is evidence that DCA concentrates in rat myocardial mitochondria (Smith et al., 1992; Kerbey et al., 1976).

4.5.4. Neurotoxicity

Neurologic symptoms and morphologic changes in the nervous system have been reported in humans, dogs, and rats at comparable doses (when expressed as mg/kg). Reversible

peripheral neuropathy has been noted in humans after several months of daily, oral doses of 50 to 100 mg/kg-day (Moore et al., 1979; Spruijt et al., 2001; Stacpoole et al., 1998a). Beagle dogs treated orally with DCA developed partial paralysis of the hind limbs at doses of 72 mg/kg and above (Cicmanec et al., 1991; Katz et al., 1981). Morphologic alterations in the CNS, including vacuolization of the myelinated white tracts in the cerebellum, cerebrum, and spinal cord, were observed in dogs at doses of 12.5 to 72 mg/kg-day. In rats, dose-limiting toxicity is associated with hind limb paralysis and peripheral neuropathy (Katz et al., 1981; DeAngelo et al., 1996). Brain lesions characterized by the vacuolization of white tracts have been noted at doses of 125 to 2000 mg/kg-day (Katz et al., 1981). Focal vacuolation and gliosis were present in the forebrain and brain stem of rats treated with 1,100 mg/kg-day (Bhat et al., 1991). Progressive changes in gait were observed in 2 strains of weanling rats exposed, via drinking water, to 16, 89 or 173 mg/kg-day DCA (F344 strain) and 17, 88 or 192 mg/kg-day DCA (LE strain) for 13 weeks (Moser et al., 1999). In addition, hind limb grip strength was decreased throughout exposure in LE rats treated with 88 or 192 mg/kg-day (but no dose-response relationship was evident) and in F344 rats treated with 173 mg/kg-day DCA. Also, tremor, hypotonia, and inhibition of pupil reflex were observed in both high-dose strains. Moser et al. (1999) provided information pertaining to the potency of DCA in drinking water vs. oral gavage, reversibility of effects, and strain and age differences. To date, no signs of neurologic toxicity or morphologic changes of the nervous system have been reported in DCA-treated mice.

4.6. SYNTHESIS AND EVALUATION OF CANCER EFFECTS AND MODE OF ACTION

4.6.1. Data Summary

No epidemiological investigations of the carcinogenicity of DCA in humans have been performed. However, there have been a number of studies on cancer risk in humans who ingest chlorinated drinking water (which may contain DCA as a disinfection by-product). A number of these studies show a weak correlation between exposure to chlorinated drinking water and risk of bladder cancer. However, available data are not sufficient to establish a causal relation between the ingestion of chlorinated water and the risk of developing cancer (U.S. EPA, 1998d). Further, even if data ultimately establish an increase in cancer risk that is attributable to the ingestion of chlorinated water, it cannot be concluded from these studies that DCA per se is carcinogenic in

humans, since chlorinated water contains a wide spectrum of potentially carcinogenic disinfection by-products.

In animals, there have been a number of independent studies investigating aspects of the carcinogenicity of DCA. Among these studies, statistically significant increases in hepatic carcinomas alone and/or hepatic carcinomas plus adenomas was seen in: (1) all male B6C3F_1 mouse studies (Herren-Freund et al., 1987; Bull et al., 1990; DeAngelo et al., 1991, 1999; Daniel et al., 1992; Ferreira-Gonzalez et al., 1995); (2) all but the last cited female B6C3F_1 mouse study (Pereira and Phelps, 1996; Pereira, 1996; U.S. EPA, 1991b; Bull et al., 1990); and (3) in three F344 rat studies (DeAngelo et al., 1996; Richmond et al., 1995). Based on these findings, it is recognized that DCA is hepatocarcinogenic in male mice and rats, and that exposure to high concentrations of DCA in drinking water can significantly increase the incidence of liver adenomas and/or carcinomas. Exposure levels causing increased incidence tumors in animals range from 0.5 to 5 g/L. However, concentrations as low as 0.05 g/L (8 mg/kg-day) increase the multiplicity of tumors in male mice (DeAngelo et al., 1999).

The induction of liver tumors in mice is a widely debated endpoint in cancer bioassays. This is particularly true for male B6C3F_1 mice, which are especially susceptible to developing liver tumors from a variety of chemical insults. However, the positive findings in female mice and rats indicate that the carcinogenicity of DCA is not restricted to male mice, and that the tumorigenic response is likely to be relevant across different species. The fact that DCA induces liver tumors in the rat at lower doses than in the mouse also strengthens the overall weight of evidence for DCA's tumorigenicity.

Some support for the relevance of the hepatic tumors observed in rodents to humans is provided by the fact that liver tumors are sometimes a consequence of glycogen storage disease (VI) and hereditary tyrosinemia I. As mentioned previously, several of the hepatic manifestations of DCA-exposure in rodents (liver enlargement and glycogen accumulation) are similar to the consequences of untreated glycogen storage disease (VI) while the DCA inhibition of GSTZ produces increased concentrations of the same intermediary tyrosine metabolites that are increased in tyrosinemia I. In addition, the work of Carter et al. (2003) seems to support a multifactorial origin for DCA-induced liver tumors in animals. Examination of the tissues from the mice used in the DeAngelo et al. (1999) study suggests that cancerous liver tumors can originate from eosinophilic, dysplastic, and basophilic or clear cells of exposed animals. One or more of these origins may be relevant to humans.

As with many carcinogens, the duration of exposure is an important determinant of the magnitude of the tumorigenic response. Several studies in male and female B6C3F$_1$ mice found multiple tumors per animal with treatment concentrations of 2 g/L and above within one year (Herren-Freund et al., 1987; Bull et al., 1990; DeAngelo et al., 1991; Pereira, 1996). At this time point, the dose-response is very steep with no response observed at concentrations of 1 g/L or lower. However, lower concentrations (0.5 g/L) resulted in a hepatic tumor incidence of approximately 80% in a full two-year study in male mice (Daniel et al., 1992). This same temporal relationship occurred in the rat at doses as high as 2.4 g/L required to induce tumors at 60 weeks. Doses as low as 0.5 g/L induced liver tumors (incidence, 41%) when exposure was 104 weeks (Richmond et al., 1995).

There is a considerable increase in the internal DCA dose with drinking water concentrations between 1 and 10 g/L in cases where GSTZ is inhibited according to the Barton et al. (1999) pharmacokinetic model. The increase is coincident with the concentration associated with a statistically significant increase in cancer prevalence in the study by DeAngelo et al. (1999) and supports the hypothesis that either DCA or the alkylating tyrosine metabolites that accumulate when GSTZ is inhibited may be the causative agent (Table 4-5). However, this hypothesis does not explain the significant increase in tumor multiplicity at lower doses.

Additional support for classifying DCA as a carcinogen comes from the data base of other carcinogenic compounds such as perchloroethylene, trichloroethylene, trichloroacetic acid, and chloral hydrate. Each of these compounds produce DCA as a metabolite (IARC, 1979; Lash and Parker, 2001). However, DCA production alone is unable to account for the carcinogenic properties of the more highly halogenated two-carbon precursor compounds.

Table 4-5. Drinking water exposures, cancer response and simulated internal dose metrics

Drinking Water Concentration (g/L)	Daily Dose mg/kg-day	Modeled AUCL mg-hr/L	Carcinoma Prevalence (%)	Carcinoma Multiplicity
0	0	0	26	0.28
0.05	8	0.041	33	0.56*
0.5	84	0.72	48	0.68*
1	168	15.8	71*	1.29*

2	315	417	95*	2.47*
2.5	429	1064	100*	2.90*

* Statistically different from control.
Source: Adapted from DeAngelo et al. (1999).

4.6.2. Potential Mode of Carcinogenicity

While a number of studies provide some information on the mode of action by which DCA may increase cancer incidence in animals, none of them provide a satisfactory mode of action for the carcinogenicity of DCA. Note that it is not necessary to assume that only one mode is operative, and the possibility exists that different modes may be acting in different species, or even in the same species at different doses. The most likely modes of action for the carcinogenic activity of DCA are briefly summarized below.

Mutagenicity and Genotoxicity

The genotoxicity database on DCA has been extensively reviewed by several scientific organizations including IARC (1995), ILSI (1997), WHO (2000), and EPA (1998c). Based on an evaluation of data available at the time, IARC (1995) and ILSI (1997) reached independent conclusions that DCA was not genotoxic. A more recent review by WHO (2000) concluded that, although there is some evidence that DCA is genotoxic at high concentrations, these effects are not likely to be involved in the mechanism of DCA tumorigenesis. In another recent review, Moore and Harrington-Brock (2000) concluded that the available genotoxicity data indicate that DCA is very weakly mutagenic. In contrast, the National Center for Environmental Assessment (U.S. EPA, 1998c) concluded that available data indicate that DCA is a direct-acting genotoxic agent. This conclusion is based on recent studies conducted at the National Health and Environmental Effects Research Laboratory (DeMarini et al., 1994; Fuscoe et al., 1996; Leavitt et al., 1997; Harrington-Brock et al., 1998) that reveal DCA's ability to cause mutational damage, induce point mutations in DNA and cause chromosomal aberrations. Note that several of these newer studies were published after the IARC and ILSI evaluations.

The majority of evidence indicates that DCA is a weak mutagen, inducing mutations and chromosome damage *in vitro* and *in vivo* assays predominantly at high concentrations. Nevertheless, in the absence of causal data, EPA considers it prudent to assume that DCA might be genotoxic, at least under *in vivo* exposure levels that are associated with detectable increases

in tumor incidence (particularly at the higher doses). Whether DCA is genotoxic at lower doses (which would suggest a linear dose-response curve for cancer risk) is not known.

Cytotoxicity and Compensatory Hyperplasia

One plausible hypothesis for DCA-induced tumorigenesis is that it occurs secondary to increased cell proliferation (Ames and Gold, 1991a, b). Increasing the rate at which a cell divides increases the probability that some critical genetic error will occur or a quiescent error will be clonally expanded, potentially resulting in a transformed cell. Regenerative hyperplasia in response to cytotoxicity is one way that this can occur. As previously discussed in Section 4.5, there is evidence of liver toxicity in DCA-treated mice at doses shown to cause tumor development. However, data in the rat do not fit this paradigm. Although frank liver toxicity (necrosis) has not been reported in DCA-treated rats, there is explicit evidence in the literature to the contrary. DeAngelo et al. (1996), for example, examined the livers of the rats in all dose groups including the highest dose (2 g/L) and reported no evidence of increased hepatocellular necrosis and no increase in labeling index. These findings indicate that cell killing and regenerative hyperplasia were minimal. Subsequently, the authors reported to ILSI (1997) that there were small increases in serum enzymes associated with DCA exposure, suggesting that a low level of cell death and lysis may have been occurring. Whether this could account for the strong tumorigenic response is not clear. Subsequent studies by DeAngelo et al. (1999) detected an increased incidence and multiplicity of hepatic tumors in male mice that displayed no apparent cytotoxic or regenerative response to DCA. It also seems reasonable that if necrosis/cytotoxicity were an important precursor to DCA hepatocarcinogenesis, then the rat, which demonstrates far less liver toxicity, should by extension be less susceptible to DCA tumorigenicity. As previously described, this is not the case (DeAngelo et al., 1991, 1996).

Utilizing magnetic resonance imaging techniques, Miller et al. (2000) demonstrated that DCA can affect growth rates of liver tumors. In particular, the results suggest that the primary effect of DCA in tumor induction is mediated through accelerated growth of spontaneously-initiated cells. To some extent this conclusion is supported by studies suggesting that DCA influences the structure of H-*ras* protein (Anna et al., 1994; Velazquez, 1995; Ferreira-Gonzalez et al., 1995; Schroeder et al., 1997) and the concentrations of several nuclear transcription factors (Stauber et al., 1998; Stauber and Bull, 1997; Pereira et al., 2001).

Several chemical carcinogens have been shown to induce mouse liver tumors with specific point mutations at codon 61 of the H-*ras* proto-oncogene. This mutation also occurs with very high frequency in spontaneously-derived liver tumors (~50%). Interestingly, in DCA-induced liver tumors, the frequency of *ras* mutations was the same as the frequency of *ras* mutations in the spontaneously-derived tumors of male mice (Anna et al., 1994). However, the spectrum of mutations observed in hepatic tumors was different between control and DCA-treated animals. Apparently, DCA treatment resulted in a decrease in the frequency of AAA mutations in H-*ras* with a corresponding increase in CTA mutations. The toxicological significance of this finding is not clear. Anna et al. (1994) interpreted these observations to suggest that oncogene activation was not the primary mechanism of DCA action, and that DCA may act by providing a selective growth advantage to hepatocytes bearing this type of mutation in the H-*ras* proto-oncogene.

It is clear that sufficiently high doses of DCA can cause cytolethality and regenerative hyperplasia in the liver of exposed mice, and that this response occurs in some dose groups in which a tumorigenic response is also observed. However, data from mice exposed to lower doses (DeAngelo et al., 1999) and data from rats (DeAngelo et al., 1996) indicate that this response is not required for tumorogenesis. On this basis, it is concluded that the mechanism is unlikely to completely account for the tumorigenic response, at least at doses <0.5 g/L that do not produce clear hepatotoxicity.

Peroxisome Proliferation

Increased number and/or size of hepatic peroxisomes (peroxisome proliferation) is a common finding in the livers of rodents treated with some types of hepatocarcinogens. The proliferation is regulated by a class of nuclear receptors known as peroxisome proliferator-activated receptors (PPARs) which are believed to mediate at least some of the effects reported for hepatocarcinogens, including the initiation of certain cellular events leading to transformation (U.S. EPA, 1998c). At this time, however, the precise role that increased peroxisome proliferation or PPARs plays in the actual induction of tumor formation is not clear (U.S. EPA, 1998c). Interestingly, significant species differences exist in the expression of various PPARs, and are especially prevalent between rodents and humans (U.S. EPA, 1998c). Available data suggest that humans are less responsive to a variety of peroxisome proliferators than are rats and mice. This has generated some controversy over whether peroxisome proliferators are carcinogenic in humans.

DCA has been shown to be a weak peroxisome proliferator in mice and rats (DeAngelo et al., 1998, 1999; Daniel et al., 1992; Mather et al.,1990). Transient transfection studies demonstrate that DCA activates PPARs (Zhou and Waxman, 1998). Dichloroacetic acid has also been shown to activate mouse and human PPARs with similar receptor sensitivity (Maloney and Waxman, 1999). However, the relevance of this finding to DCA tumorigenesis is not fully understood. Recently, Thai et al. (2003) found no activation of the PPARα gene in mouse liver tissues from the DeAngelo et al. (1999) animals using a microarray containing this gene. Accordingly, there is some inconsistency in the data base.

While one of the effects attributed to PPARs is the suppression of c-*Jun* activity and expression (Sakai et al., 1995), several recent studies demonstrate that DCA-induced tumors are c-*Jun* positive (Stauber and Bull, 1997; Stauber et al., 1998). This finding is inconsistent with a role for PPARs in DCA-induced tumorigenesis. It has also become apparent that DCA induces hepatic tumors in rodents at doses that are significantly below those required to induce significant peroxisome proliferation (DeAngelo et al., 1999). Collectively, these observations suggest that peroxisome proliferation is not likely to be important in the tumorigenicity of DCA.

Tumor promotion and alterations in cell replication and death

Several sets of observations suggest that DCA may be acting through a tumor promotion mechanism. For example, Stauber et al. (1998) demonstrated that DCA increased cell proliferation of c-*Jun* positive hepatocytes *in vitro*. The investigators treated isolated hepatocytes from neonatal mice with DCA and plated the cells to allow them to form colonies. While exposure of the cells to 0.5 mM DCA did not cause any cytotoxicity, it significantly increased colony formation over controls. Interestingly, the colonies that were induced by DCA were c-*Jun* positive. This is noteworthy because this is the same phenotype observed in DCA-induced liver tumors in mice exposed to DCA *in vivo* by Stauber and Bull (1997). The authors then pretreated the animals for 2 weeks with DCA in their drinking water and repeated the experiments. Dichloroacetic acid again induced c-*Jun* positive colony formation, but it only required 0.02 mM DCA for the same degree of induction. This observation indicates that DCA provided a selective growth advantage for (promoted) hepatocytes with a specific phenotype. Dichloroacetic acid has also been reported to alter cell replication rates in other assay systems, but in a complex manner. *In vivo*, exposure of cells to DCA stimulates cell proliferation at low doses in the short term, but increasing doses and chronic exposure appears to sharply inhibit

hepatocyte replication (U.S. EPA, 1998c). In normal hepatocytes (c-*Jun* negative), *in vitro* DCA administration consistently inhibits hepatocyte replication (Pereira, 1996; Carter et al., 1995). In contrast to normal cells, hepatocytes from DCA-induced liver tumors (*c-Jun* positive) are resistant to the inhibitory effects of DCA on cell proliferation (Stauber and Bull, 1997).

Data from Pereira and Phelps (1996) indicate that MNU-initiated female mice exposed to DCA in drinking water at a concentration of 20 mM exhibited a statistically significant increase in adenomas (compared to controls) when measured after 31 and 52 weeks of exposure. Herren-Freund et al. (1987) also examined DCA promotion using ENU as an initiator and doses of 400 or 1,000 mg/kg-day DCA. In mice treated with ENU and DCA, the incidence of hepatocellular carcinomas was 66% (400 mg/kg-day) or 78% (1,000 mg/kg-day), while in mice treated only with DCA at 1,000 mg/kg-day the carcinoma incidence was 81%. Thus, at high doses DCA is able to act as a complete carcinogen. This conclusion is supported by the results of DCA bioassays (Daniel et al., 1992; DeAngelo, 1991, 1996, 1999).

Chen (2000) proposed a biologically-based dose-response model for liver tumors induced by TCE and DCA. The model incorporated parameters pertaining to initiation rate, proliferation rate, conversion rate, probability of tumor progression, and death rate. A stochastic model was used to predict tumor response in TCE bioassays on the basis of its metabolite, DCA alone. The modeling results suggest that DCA may be responsible for most, if not all, TCE-induced carcinomas. Adenomas, hyperplastic nodules and other tumors were not considered in the dose-response modeling. Dosimetry was based on an unpublished PBPK model. However, in modeling liver DCA concentrations generated from trichloroethylene metabolism, Barton et al. (1999) concluded that the doses of DCA formed from trichloroethylene could not account for the tumorigenic properties of this compound.

Other Potential Mechanisms

Hypomethylation

Mammalian DNA contains the methylated base 5-methylcytosine. While the extent of DNA methylation is known to play a role in gene expression (Stroger et al., 1993), decreases in DNA methylation levels is a frequent finding in tumors and is considered to be a key factor in expanding clones and precancerous cells during neoplastic progression (Counts and Goodman, 1994, 1995). Additionally, the level of methylated DNA is lower in chemically-induced liver tumors than in normal liver tissue (Lapeyre and Becker, 1981).

Recent studies by Tao et al. (1996) demonstrate that DCA treatment decreases 5-methylcytosine levels in hepatic DNA from treated compared to control mice. Methylation of cytosine in the promoter region of genes regulates mRNA expression including that of the proto-oncogenes, c-*Jun* and c-*myc* (Tao et al., 1998, 2000). These proto-oncogenes participate in the control of cell proliferation. Cells from DCA-induced tumors have been identified as c-*Jun* positive (Stauber and Bull, 1997). Increased levels of mRNA and protein for c-*jun* and c-*myc* genes have been reported in liver and liver tumors from mice treated with DCA. Decreased methylation in the promoter regions of the c-*jun* and c-*myc* genes and increased levels of associated mRNAs and proteins were reported in the livers of mice exposed to DCA. While this observation is consistent with the hypothesis that DCA might increase tumor risk by inhibiting DNA methylation, the actual importance of this event in mediating the tumorigenic response to DCA is not known.

Conclusions Regarding Cancer Mode of Action

There are numerous questions that remain unanswered about the toxicity of DCA; many of which relate to carcinogenicity and prevent identification of a single mode of action as the only or most important pathway leading to cancer. The number of metabolic pathways affected by DCA and species differences in metabolism are still not known, nor has the ultimate toxic substance been identified. Examination of the liver tissues from animals with carcinogenic tumors suggest that the tumors can originate from several different cell lines and through more than one pathway. The impact of DCA inhibition of GSTZ and other enzymes is incompletely characterized and may be important based on the observed tendency for hepatic tumor development in humans with hereditary tyrosinemia I or glycogen storage disease VI. The

genotoxicity data for DCA are internally inconsistent, and there is little basis for judging whether genotoxic effects - including alterations in the genetic messages for various proto-oncogenes - are important in the carcinogenic response, and if so, whether the dose-response curve for genotoxic effects is linear or nonlinear. If DCA is acting as a promoter, it is possible that the dose-response curve might be linear. However, although Pereira and Phelps (1996) found some evidence for promotion, the mechanism for DCA-induced promotion is not known and so the shape of the dose-response curve is uncertain.

The importance of these issues regarding the mechanism and shape of the dose-response curves for genotoxicity and carcinogenicity are highlighted by comparing the concentrations of DCA in water that are carcinogenic in animals (0.05 to 5 g/L) with those that are commonly observed in chlorinated drinking water (10 to 100 μg/L) (U.S. EPA, 1994a; IARC, 1995). Thus, concentration values are about 4-5 orders of magnitude lower in drinking water than were used in experimental studies in animals. This difference is further magnified by the lower water intake per unit body weight of humans (approximately 0.03 L/kg-day) compared to rodents (about 0.1-0.2 L/kg-day).

4.6.3. Cancer Characterization

Previous Classification

EPA performed a cancer weight-of-evidence review for DCA in 1994 (U.S. EPA, 1994d), that was updated in 1996 (IRIS, 1996). The reviews classified DCA as a Group B2 (probable human carcinogen) in accordance with the 1986 EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a).

In 1995, IARC concluded that, based on the data available at that time, "DCA is not classifiable as to its carcinogenicity to humans," and placed DCA in the IARC Group 3 category. In 2002, IARC classified DCA in Group 2B (possibly carcinogenic to humans) based on sufficient evidence of carcinogenicity in experimental animals and inadequate evidence of carcinogenicity in humans. It is important to keep in mind that at the time of the first IARC evaluation in 1995, there was no information regarding positive carcinogenicity responses in the rat.

Current Characterization of DCA Carcinogenesis

Based on current data and the lack of conclusive data regarding the mode of action of DCA at environmentally relevant doses, DCA is considered *likely* to be carcinogenic in humans (U.S. EPA, 1999, 2003). This assessment is based on the strength of the evidence in animal bioassays. In particular, there are a number of independent studies reporting: consistently positive results at roughly comparable doses, site concordance for tumor formation between two species, consistent observations in different species and sexes, clear evidence of a dose-response relationship, and no clear data supporting a cohesive mode of action.

4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

While no data were located to establish that any particular human subpopulation is likely to be especially susceptible to the toxic effects of DCA, the toxicity of DCA appears to be related to the ability of the body to clear parent DCA by metabolism (Lukas et al., 1980; Cicmanec et al., 1991). Thus, individuals who have low activity GSTZ isozymes, or isozymes that are particularly vulnerable to inhibition by DCA might be more susceptible than the general population. Available data from a population with a Caucasian-European lineage suggest that individuals with GSTZ 1b-1b, 1c-1c, and 1d-1d isozymes might have a different response to DCA than those with 1a-1a isozyme (Blackburn et al., 2000, 2001). Individuals with glycogen storage disease (an inherited deficiency or alteration in any one of the enzymes involved in glycogen degradation) represent another group that may be more susceptible to DCA toxicity. There is some evidence that alterations in glycogenolysis precede the development of many tumor types (Bannasch, 1986). The dose-response for DCA-induced effects on hepatic glycogen is in the same range as that required for inducing liver tumors (Bull, 2000). In addition, DCA is thought to be metabolized by at least one free radical generating pathway, and peroxidation has been proposed as a mechanism for DCA toxicity. Thus, it is possible that catalase-deficient individuals may also experience increased risk.

Individuals with hyperoxaluria Type 1, a rare genetic disorder, would be susceptible to elevated glyoxalate originating from DCA. While data are unavailable regarding the prevalence of this rare disorder in the United States, data from France indicate that the prevalence is 1.05 per 1 million individuals (Cochat et al., 1995). In this condition, the inability to convert glyoxyalate to glycine leads to the formation and excretion of oxalate (Montgomery et al., 1990).

Specific information on whether children are more susceptible than adults to the effects of DCA are not available. At this time there are no indications that there are unique GST Zeta isoforms expressed in the fetus or neonate. GST zeta is expected to be active in neonates, infants, and children because of its role in catabolism of tyrosine, an amino acid, which is present in milk other protein foods and needed for growth and development. Accordingly, GST zeta alone is unlikely to play a direct role in childhood susceptibility.

In female rats, DCA exposure during gestation resulted in the impairment of fetal maturation and soft tissue anomalies (primarily of cardiac origin) indicating that the developing fetus is susceptible to DCA-induced toxicity (Smith et al., 1992). Data collected by Moser et al. (1999) provide limited evidence for increased susceptibility of rats to DCA-induced neurotoxicity when exposures begin shortly after weaning.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

The oral reference dose (RfD) is based on the assumption that thresholds exist for certain toxic effects (e.g., cellular necrosis) and is expressed in units of mg/kg-day. The RfD is an estimate (with uncertainty spanning perhaps one order of magnitude) of a daily exposure to humans (including sensitive individuals) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

5.1.1. Methods of Analysis

Data on the noncancer effects of DCA were used to estimate RfD values using two different approaches: (1) the traditional NOAEL-LOAEL approach (Section 5.1.2.), and (2) the Benchmark Dose (BMD) modeling approach (Section 5.1.3).

5.1.2. NOAEL/LOAEL Approach

Data Summary

Figure 5-1 graphically presents the NOAELs and LOAELs from studies that examined the noncancer effects of DCA. NOAEL values are shown by open symbols and LOAEL values are shown by closed symbols. A table that summarizes the data displayed in Figure 5-1 is included as Appendix B. Some of the studies from Figure 5-1 were not considered suitable for quantitative risk assessment because there was no LOAEL or there were too few dose groups to permit an assessment of dose-response. The key studies that were considered for quantitative risk assessment are summarized in Table 5-1.

Oral exposure levels of 12.5-200 mg/kg-day have been demonstrated to cause all of the characteristic noncancer effects in animals, and most of these effects (impacts on metabolism, neurotoxicity, liver effects) have also been observed in humans at similar doses. Based on the general similarity in the effect levels reported for each response category, it is not apparent that any one effect occurs at a clearly lower dose than the others, and that one type of effect should be considered critical.

Selection of Principal Study and Critical Endpoint

The study of Cicmanec et al. (1991) identifies the lowest LOAEL (12.5 mg/kg-day) that has been determined to date (Table 5-1). In this study, beagle dogs were administered oral doses (12.5 to 72 mg/kg-day) of DCA in capsules for 90 days. Adverse effects noted in the low-dose group (12.5 mg/kg-day) included mild to severe testicular degeneration in four of five males, along with mild to moderate hepatic vacuolization and mild vacuolization of the myelinated white tracts of the cerebrum and cerebellum in males and females. This study is supported by the findings of Katz et al. (1981), who noted marked testicular degeneration and myelin vacuolization in dogs administered oral doses of 50 mg/kg-day or higher for 13 weeks. While testicular effects have not been noted in humans, this effect has not been monitored in the human population. Data from humans administered DCA as a pharmaceutical indicate that doses of 25-50 mg/kg-day produce neurological effects, including sedation and peripheral neuropathy (Moore et al., 1979; Spruijt et al., 2001; Stacpoole et al., 1998a). Based on these considerations, the study of Cicmanec et al. (1991) is judged to identify a LOAEL that is likely to be appropriate for humans, and is selected as the principal study. The effects of concern are testicular degeneration accompanied by mild histopathological alterations in the liver and brain.

Table 5-1. Summary of noncancer studies considered for benchmark modeling

Endpoint	Species	Reference	Duration	Dose Grps.[a]	NOAEL	LOAEL	Effect
Metabolism	Rat	Katz et al., 1981	3 months	4		125	Decreased serum lactate and glucose
	Dog	Katz et al., 1981	13 weeks	4		50	Decreased serum metabolites
Hepatic	Mouse	Sanchez and Bull, 1990	2 weeks	4	57	190	Increased glycogen, focal necrosis
		Kato-Weinstein et al., 1998	2 weeks	6	20	100	Increased glycogen
		Bull et al., 1990	1 year	3		140	Increased glycogen
		DeAngelo et al., 1991	60-75 weeks	3	7.6	77	Increased liver weight
	Rat	Mather et al., 1990	3 months	4	3.9	35.5	Increased liver weight
		Katz et al., 1981	3 months	4		125	Increased liver weight
		Smith et al., 1992	gestation	4	14	140	Increased liver weight
		Toth et al., 1992	10 weeks	4		31	Increased liver weight
	Dog	Cicmanec et al., 1991	3 months	4		12.5	Increased liver weight, inflammation
Neurologic	Rat	Katz et al., 1981	3 months	4		125	Histological brain lesions
	Dog	Cicmanec et al., 1991	3 months	4		12.5	Vacuolar changes in brain
		Katz et al., 1981	13 weeks	4		50	Vacuolar changes in brain
Reproductive	Rat	Smith et al., 1992	gestation days 6-15	4	14	140	Decreased fetal wt, increased resorptions
		Linder et al., 1997	2 weeks	5		54	Impaired sperm formation
		Toth et al., 1992	10 weeks	4		31	Impaired sperm formation
		DeAngelo et al., 1996	100 weeks	3	3.6	40.2	Increased testicular weight
	Dog	Katz et al., 1981	13 weeks	4		50	Prostate atrophy, testicular changes
		Cicmanec et al., 1991	3 months	4		12.5	Testicular degeneration

[a] Number of dose groups, including control
NCV = Nerve conduction velocity

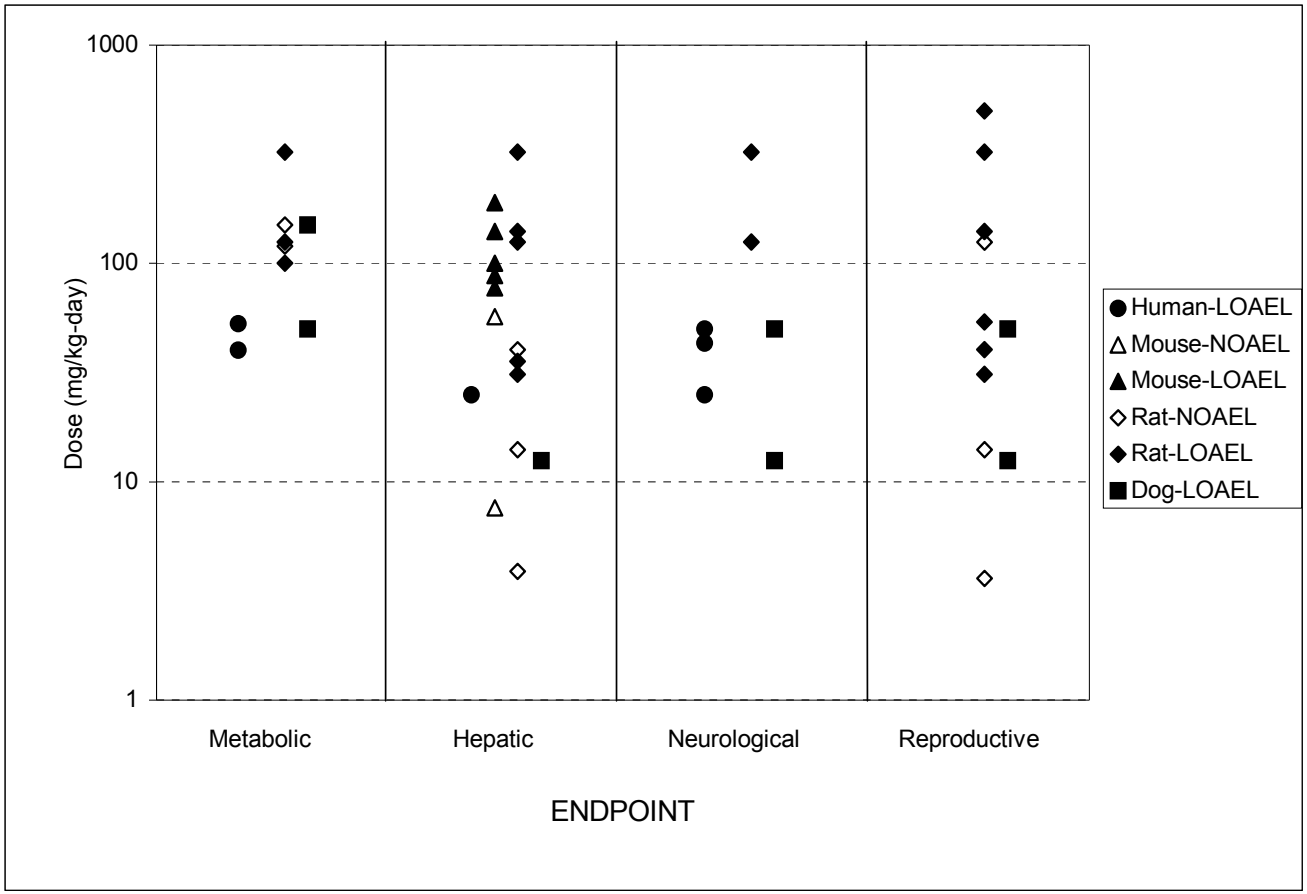

Figure 5-1. Summary of noncancer effects of DCA.

Calculation of the NOAEL-LOAEL Based RfD

Based on the LOAEL of 12.5 mg/kg-day identified by Cicmanec et al. (1991), the oral RfD for DCA is calculated as follows:

RfD = (12.5 mg/kg-day) / (3000) = 0.0042 mg/kg-day (Rounded to 4E-03 mg/kg-day)

where:

12.5 mg/kg-day = LOAEL, based on lesions observed in the testes, cerebrum, cerebellum, and the liver of dogs exposed orally (via gel capsules) to dichloroacetic acid for 90 days.

3,000 = Uncertainty factor. This uncertainty factor includes a factor of 10 to account for potential inter-human variability in susceptibility to DCA, a factor of 3 to account for extrapolation from animal data to humans, a factor of 10 to account for use of a LOAEL, a factor of 3 to account for the use of a less-than-lifetime study in which frank effects were noted, and a factor of 3 to account for deficiencies in the database.

A factor of 10 was applied for intrahuman variability because of the observation that the most frequent human GSTZ variant (GSTZ 1c-1c) is one that has a low activity toward DCA and is also impacted by DCA inhibition to a greater extent than the most active, but less frequent human variant (GSTZ 1a-1a). Accordingly, one might expect poor clearance of DCA from human plasma via oxidative dechlorination when exposure is continuous.

A threefold factor was applied for interspecies variability. There are several reasons for this choice and the resulting partial reduction of the UF from the default of 10. First, death occurred at a dose of about 75 mg/kg-day DCA (90 day study) in 3/10 and 1/3 dogs after 51 and 74 days of dosing and 50 days of dosing, in the principle study by Cicmanec et al. (1991) and the study by Katz et al. (1981) respectively. Conversely, Stacpoole et al. (1998b) reported on cases of five children with lactic acidosis who received 25-60 mg/kg-day orally for two months to four years without clinical signs of DCA toxicity (elevation of liver enzymes and neuropathy). Although two of the children died during treatment, death was the result of infection and not from the lactic acidosis or DCA treatment. Annual mortality in patients with congenital lactic acidosis, even with treatment, is 20%.

Additional support for this conclusion is provided by the fact that metabolic effects of DCA on serum lactate and glucose in dogs (Katz et al., 1981; Ribes et al., 1979) parallel those in humans (Stacpoole et al., 1998a, b). Stacpoole et al. (1998b) reported that lactate concentrations decreased by at least 20% within 24 hours after oral doses of 25 to 100 mg/kg in humans. In the study by Katz et al. (1981), there was an approximate 40% reduction in serum lactate concentrations of dogs (male and female) after 13 weeks of exposure to 50 mg/kg-day DCA.

Limited toxicokinetic data suggest that dogs metabolize DCA at a slower rate than humans and rodents supporting the concept of their increased sensitivity (Section 3.3; Lukas et al, 1980; Curry et al, 1991; Lin et al, 1993; Larson and Bull, 1992; James et al, 1998). A single intravenous dose of 100 mg/kg in two dogs lead to peak plasma levels that were twice as high as the same levels in rats (Lukas et al., 1980).

Lastly, the structure of GST Zeta appears to be highly conserved across species making it unlikely that the metabolic differences in humans will differ from dogs by a full order of magnitude, also taking into consideration that the full UF=10 has been applied for intrahuman variability. Under these circumstances an interspecies uncertainty factor of 3 rather than the default 10-fold value is justified.

The factor of 10 for the use of a LOAEL is justified by the observed effects of DCA on the nervous system in sensitive humans (those under treatment for lactic acidosis and other disorders) at doses of 25-50 mg/kg-day. These doses are within the same order of magnitude as the LOAEL in the Cicmanec et al. (1991) study and the LOAEL for neutotoxicity in F-344 and LE rats in adult and weanling rats in the Moser et al. (1999) study. There are no human data on testicular effects from DCA.

Threefold factors were applied for both the use of data from a less-than-lifetime study and database inadequacies. The database for DCA lacks a multi-generation study of reproductive toxicity and a developmental neurotoxicity study, thus, meriting an uncertainty factor of 3 for database insufficiency. Otherwise the database is comprehensive with information from subchronic and lifetime animal studies, studies in three animal species, and over 25 years of experience with the use of DCA as a experimental pharmaceutical in the treatment of several human disorders.

The richness of the data base does not abrogate all concern associated with using a subchronic study as the basis of the RfD, but is sufficient to reduce the uncertainty factor from a 10 to a 3. The neurological effects of DCA in the principal study are a concern as is the fact that there are no data on the mechanism for the observed neurological or testicular effects. Neurological effects were seen in humans and other animal species (rats, dogs) at doses comparable to the LOAEL in the Cicmanec et al. (1991) study. They were severe enough in human patients to alter the clinical treatment regime. About 20 to 50% of patients with lactic acidosis experience sedative effects with single and repeated oral or intravenous doses of 25 to 50 mg/kg-day. The effects were reversed with the withdrawal of DCA, but in some patients reversal was slow (Stacpoole et al., 1998a). The effects on the nervous system seen in dogs involved vacuolization of myelin. This observation can be mechanistically linked to the decreased nerve conduction velocity observed in human subjects (Spruijt et al., 2001) since nerve impulses travel faster in myelinated nerves. Therefore, the use of an uncertainty factor of 3 to extrapolate from subchronic to chronic exposures is appropriate. There are no data that permit an assessment of the clinical progression of the neurological effects. The data on testicular effects could be more robust, but are mitigated by the lack of testicular histopathology in the DeAngelo et al. (1996) rat cancer study. Rats are susceptible to testicular effects as a result of DCA exposure (Linder et al., 1997; Toth et al., 1992), but the data indicate they are less sensitive to this effect than dogs.

5.1.3. Benchmark Dose Approach

Selection of Data Sets for Modeling

It is apparent from Figure 5-1 that DCA produces effects on metabolism, the liver, and the nervous system at doses between 10 and 100 mg/kg-day in rats, mice, dogs and humans. Effects on the reproductive system are seen in rats, mice, and dogs at the same doses. Some observed effects such as the metabolic changes are biomarkers of exposure and others are unequivocally adverse at doses between 10 and 100 mg/kg-day. The data from these studies were evaluated for their suitability in establishing a protective dose-response curve using benchmark dose analysis following the criteria outlined in Table 5-2. Studies were eliminated from consideration for benchmark modeling because of the following reasons:

- there were only one or two doses;
- there was no LOAEL;

- the LOAEL exceeded 200 mg/kg-day (a LOAEL of 200 mg/kg-day is more than 10 times the lowest LOAEL of 12.5 mg/kg-day seen in the Cicmanec et al. (1991) study); and
- the effects were not definitively adverse.

All of the studies that were considered as potential candidates for modeling are included in Table 5.1. Among the studies that were included by the quantitative criteria outlined above, several were excluded from modeling on the basis of semi-quantitative or qualitative criteria, as follows:

- The study by Katz et al. (1981) on the effect of DCA on serum metabolite levels in dogs was not selected because the doses administered resulted in markedly reduced appetites at all doses, and both sexes exhibited dose-dependent weight losses. Thus, effects on serum metabolite levels might simply be secondary to decreased food intake. In addition, one of three mid-dose female dogs and one high-dose male dog died during the study. Thus, the number of animals surviving at the end of the study is too small to allow reliable BMD modeling.

- Data from the study by DeAngelo et al. (1991) demonstrating increased liver weight was not used because the increase was due to hepatic tumor growth. Increased liver weight, in this case, is not an appropriate indicator of noncancer effects.

- The data set on liver histopathological lesions reported by Cicmanec et al. (1991) was not retained because most of the lesions were ranked as mild and are not likely to be of significant toxicological concern.

- Data on the effects of DCA on testicular weight in rats (DeAngelo et al., 1996) were not used. Although DCA caused a slight, but significant increase in absolute and relative testes weight at 40.2 mg/kg-day, there were no accompanying histopathological effects in these tissues. In a second study in male rats performed by the same investigators, a significant decrease in absolute (but not relative) testes weights was observed at the single dose used (139 mg/kg-day). When considered together, the data indicate that increases in testes weight observed at a lower dose range might reverse at higher doses. The data were considered inappropriate for modeling. In addition, the endpoint was not deemed to be the most sensitive because no histopathological effects were noted.

- Data on vacuolar changes in brain reported by Cicmanec et al. (1991) were not utilized because there was no consistent dose-response trend in the data.

Table 5-2. Criteria for selecting studies appropriate for BMD modeling[a]

Category	Criterion	Rationale	Possible Exceptions
Quantitative	The chosen study should have at least 2 dose groups, plus a control group.	A study with only 1 dose group and a control does not provide enough data to define the shape of the dose-response curve.	If the one exposure group yields a response near the BMR, a suitable estimate of the BMD may be possible.
	The LOAEL from the chosen study should be within a factor of 10 of the lowest LOAEL from other studies.	Studies that identify LOAEL values more than 10-times the lowest LOAEL are very unlikely to be based on the most sensitive endpoint and are unlikely to yield the lowest BMD.	
	The NOAEL from the chosen study should not exceed the lowest LOAEL from other studies.	Studies that identify NOAEL values that exceed the lowest LOAEL are very unlikely to be based on the most sensitive endpoint and are unlikely to yield the lowest BMD.	If the study defines a reasonable dose-response trend below the NOAEL, but the NOAEL is elevated because of lack of statistical power, the study might be worthy of evaluation.
Semi-Quantitative	The LOAEL should not be a near-maximal adverse response.	If the response in the lowest dose group is at the high end of the dose-response curve, the data will not provide information on the shape of the curve at doses that produce responses near the BMR, and BMD estimates will be unreliable.	If the shape of the dose-response curve is very steep, then the dose-response curve will be reasonably constrained even if the response at the low-dose group is well above the BMR.
	The data should have a clear dose-response trend, preferably smoothly graded (monotonic).	If no clear dose-response trend is apparent, the data are not suitable for establishing a dose-response curve.	
Qualitative	The endpoint for which there is dose-response data should have clear toxicological relevance.	There is little basis for setting a BMR (and, hence, estimating a BMD) for endpoints which are not easily interpretable in terms of their toxicological significance. Endpoints which are known to be early indicators of the adverse effects of the chemical are preferred.	

[a] U.S. EPA, 2000c

- The findings of Katz et al. (1981) on histopathological changes in brain and testes were not used because no quantitative data on the severity or incidence of these effects were provided.

Based on these evaluations, the following data sets were judged to warrant BMD modeling:

- low birth weight and cardiac malformations in rats (Smith et al., 1992);
- increased relative liver weight (Mather et al., 1990);
- impaired sperm formation in rats (Linder et al., 1997);
- impaired sperm formation in rats (Toth et al., 1992); and
- testicular lesions in dogs (Cicmanec et al., 1991).

In the case of the Smith et al. (1992) study, EPA had already modeled the data using the THRESH Benchmark Dose program (U.S. EPA, 1998e). Because the original data are no longer available from the study (based on oral communication with the author), model inputs were limited to the data provided in the published paper.

The Smith et al. (1992) study was conducted in two segments: one segment utilized a DCA dose range of 900-2,400 mg/kg-day and the second utilized a dose range of 14-400 mg/kg-day. The cardiac malformation data were presented as the percent of fetuses affected per litter. The data were converted to affected fetuses per litter using the average number of fetuses per litter (calculated from the number of fetuses and the number of litters [Table 5-3]). Data from all dose groups were modeled to determine the BMD and BMDL. According to the best-fit model for the cardiovascular defects, the $BMDL_{10}$ (10% response level) was 567 mg/kg-day.

Table 5-3. Cardiovascular defects induced by DCA

Dose Group (mg/kg-day)	Mean Percent Fetuses Affected per Litter	Average Number of Fetuses per Litter	Estimated Incidence
0	0*	13	0
14	0.69	8.4	1
140	1.02	9.2	1.8
400	8.07	8.6	13.2
900	8.15	8.6	11.2
1,400	23.91	8.6	37.1
1,900	43.67	8.5	63.3
2,400	68.75	6.8	74.0

* The control data from both segments of the study have been added together.
Source: Adapted from Smith et al. (1992).

For the fetal body weights, it was necessary to use a combined mean weighted average for males and females in each dose group because the fetal body weights were reported as a continuous variable. In addition, it was necessary to estimate the mean responses of the animals and the corrected sum of squares based on the published information in order to model the data (Table 5-4). The parameters were not reported in the published version of the study. Initial modeling for the fetal body weights incorporated data from all dose groups; however, use of the control and the three lower dose groups from part two of the study provided the best fit in the low-dose range. The BMDL values for 10, 5 and 1% reductions in mean fetal body weight were 458, 259 and 60 mg/kg-day, respectively. All of these values exceed the NOAEL identified by the Cicmanec et al. (1991) study and, thus, were not considered to be suitable for derivation of the RfD.

Table 5-4. Effects of DCA on fetal body weight

Dose Group (mg/kg-day)	Combined Mean Fetal Body Weight (g)	Combined Standard Deviation	Estimated Corrected Sum of Squares
Low-Dose Segment of the Study			
0	3.58	0.22	11.81
14	3.68	0.31	20.84
140	3.54	0.21	11.27
400	3.36	0.31	22.75
High-Dose Segment of the Study			
0	3.57	0.22	13.24
900	3.06	0.22	9.39
1400	2.90	0.31	20.81
1900	2.77	0.31	19.32
2400	2.68	0.24	8.93

Source: Adapted from Smith et al. (1992).

The software employed for benchmark dose modeling of the remaining studies was BMDS Version 1.2 or 1.3.1, downloaded from EPA's NCEA web site. The data for dichotomous endpoints were fit to each of the dichotomous models provided in the software, including gamma, logistic, multi-stage, probit, quantal-linear, quantal-quadratic, and Weibull. The data for continuous endpoints were fit to each of the continuous models offered in the BMDS software (linear, polynomial, power, and Hill).

Mather et al. (1990) identified a NOAEL for the liver weight effects from a study that included four dose groups and was considered suitable for modeling (Table 5-5). Several other studies (Cicmanec et al., 1991; Kato-Weinstein et al., 1998; Toth et al., 1992) provide quantitative dose-response data on increases in absolute or relative liver weight which justifies consideration of this endpoint for modeling. The findings were attributed to increased glycogen accumulation. Glycogen accumulation may play a role in the toxicity of DCA. Significant increases in relative liver weight were also observed in a chronic-duration study in which rats were administered a time-weighted average dose of 1.6 g/L DCA (DeAngelo et al., 1996).

Table 5-5. Liver weight data set

Endpoint (rats)	Parameter	Dose (mg/kg-day)			
		0	3.9	35.5	345
Increased Relative Liver Weight	Mean ± S.E.	3.8 ± 0.10	4.08 ± 0.01	4.73 ± 0.09	6.42 ± 0.10

Source: Adapted from Mather et al. (1990).

Studies by Cicmanec et al. (1991), Katz et al. (1981), Linder et al. (1997), and Toth et al. (1992) have all identified effects of DCA on testicular histopathology and/or sperm parameters. Several of the data sets were suitable for benchmark dose modeling. Table 5-6 summarizes the dose-response data sets that were evaluated for the male reproductive system effects.

Table 5-6. Male reproductive data sets used for BMD modeling

Endpoint (rats)	Parameter	Dose (mg/kg-day)			
		0	31.25	62.5	125
Epididymal Sperm Count (10^6/g)	N Mean Stdev	19 630.3 204.8	18 582.5 137.0	18 502.6 163.5	19 367.8 91.6
Sperm Morphology (percent normal)	N Mean Stdev	20 85.1 19.2	19 86.7 16.9	17 80.4 14.1	19 58.9 16.2
Sperm Motility (percent motile)	N Mean Stdev	15 54.6 10.2	14 54.1 11.2	17 39.5 12.0	19 27.1 9.8

Source: Toth et al. (1992).

Endpoint (rats)	Parameter	Dose (mg/kg-day)					
		0	18	54	160	480	1440
Cauda Sperm Count (10^6)	N Mean Stdev	8 224 40	8 248 32	8 208 25	8 165 21	8 106 35	8 86 11
Sperm Motility (percent motile)	N Mean Stdev	8 72 12	8 74 8	8 72 9	8 41 13	8 20 17	8 6 7

Source: Linder et al. (1997).

Endpoint (dogs)	Parameter	Dose (mg/kg-day)			
		0	12.5	39.5	72
Testicular Degeneration (Incidence)	N Affected	5 0	5 4	5 5	5 5

Source: Cicmanec et al. (1991).

Results of the BMD model fitting (with the exception of the Smith et al. [1992] data discussed above) are detailed in Appendix C. Within each data set, the preferred model was selected based mainly on the quality of the model fit to the data, judged in part by the *p* value and in part by visual inspection of the fit. Models that yielded a *p* value less than 0.100 were not considered further. When more than one model gave similar quality fits, the model that yielded the lowest BMD was preferred. The results are summarized in Table 5-7.

Table 5-7. Summary of noncancer BMD modeling results

Reference	Data Set	BMR	Preferred Model (a)	*P* value	BMD mg/kg-day	BMDL mg/kg-day
Cicmanec et al. (1991)	Incidence of testicular degeneration	10% Extra risk	Quantal Quadratic	1.000	3.2	2.1
Linder et al. (1997)	Cauda Sperm count	1 Stdev	Hill	0.180	73.8	- (b)
	Sperm motility	1 Stdev	Hill	0.173	74.4	52.0
Toth et al. (1992)	Epididymal Sperm count	Point Risk	Linear	0.891	87.4	- (b)
	Sperm motility	1 Stdev	Hill	- (c)	55.8	40.4
	Sperm morphology	1 Stdev	Power	0.619	101.5	74.7
Mather et al. (1990)	Relative Liver Weight (Liver weight/body weight ratio)	1 Stdev	Hill	- (c)	3.3	3.0

(a) The preferred model is the one that fits the data best. If more than one model gave comparable fits, the preferred model is the one yielding the lowest BMD.
(b) The software could not calculate a BMDL, including an attempt to define BMR as a point risk.
(c) Chi-Square Test for fit not valid (degrees of freedom are less than or equal to 0).

As seen in Table 5-7, two of the data sets could not be adequately described by any of the continuous models: Toth et al. (1992) [sperm motility data] and Mather et al. (1990) [liver weight]. Of the remaining data sets, four (based on the data of Linder et al. [1997] and Toth et al. [1992]) yielded BMD values ranging from 74-102 mg/kg-day. In two of these cases, the software was not able to calculate a BMDL (the lower confidence bound on the BMD). In the other two cases, the BMDL values ranged from 52-75 mg/kg-day.

In contrast to these results, the data set reporting incidence of testicular lesions in dogs (Cicmanec et al., 1991) yielded a very high quality fit ($p = 1.000$), and much lower BMD and BMDL values (3.2 and 2.1 mg/kg-day, respectively) than the data from rats. However, this apparent goodness-of-fit is an artifact because none of the dose groups in this study yielded a response near the BMR, so the shape of the curve is essentially unconstrained in the low-dose range. Thus, even though the fit appears to be of high quality, both the BMD and the BMDL are judged to be unreliable.

Calculation of the BMD-Based RfD

BMD modeling of effects on sperm formation in rats (Toth et al., 1992; Linder et al., 1997) yield BMD values of 74-102 mg/kg-day. These values (based on a BMR of one standard deviation decrease from control) are substantially higher than the dose of 12.5 mg/kg-day, which yielded a high incidence (4/5) of testicular degeneration in dogs (Cicmanec et al., 1991). This suggests that rats are not as sensitive to the testicular effects of DCA as dogs, so the results from BMD modeling in rats are not considered appropriate for deriving a RfD. As noted above, even though BMD modeling of the data from dogs yields a model fit of an apparently high quality model, the numeric values of the BMD and the BMDL derived from this data set are not considered to be reliable, since the lowest dose tested yielded a high response, and there are no dose groups yielding a response near the BMR. It is recommended for BMD analyses that LOAEL values *not* be a near-maximal adverse response, which is the case for this endpoint (Cicmanec et al., 1991).

On the basis of these considerations, it is concluded that none of the available noncancer data sets provide a suitable basis for deriving an RfD for DCA via the benchmark dose modeling approach.

5.1.4. Summary of Oral RfD Derivation

Although BMD modeling often offers a number of advantages over the traditional NOAEL-LOAEL approach for deriving a reliable RfD (U.S. EPA, 1995), in this case none of the available noncancer dose-response data sets provided a suitable basis for deriving a RfD via the BMD approach. Therefore, the RfD of 4E-03 mg/kg-day derived using the NOAEL-LOAEL approach is judged to be the most appropriate assessment of chronic noncancer risk based on the current data for DCA.

5.2. INHALATION REFERENCE CONCENTRATION (RFC)

There are no data from toxicity studies of DCA that employed the inhalation route of exposure. As noted in Section 2, both the acid and salt forms of DCA have low volatility, therefore, inhalation exposure is not considered to be of concern. On this basis, an inhalation RfC for DCA is not considered necessary.

5.3. CANCER ASSESSMENT

5.3.1. Choice of Principal Studies and Cancer Endpoints

As discussed above, there are multiple studies in $B6C3F_1$ mice that establish that ingestion of DCA results in increased incidence of hepatic tumors (both hepatocellular adenomas and adenocarcinomas). Of the available studies in mice, the best one for cancer dose-response modeling is that reported by DeAngelo et al. (1999), since this study was specifically designed to establish a multi-point dose-response curve, and data are available for five dose groups plus a control group. In addition, the duration of this study spans the expected lifetime of a mouse.

It appears that the highest dose administered in this study approaches the MTD, based on the significantly elevated hepatic necrosis indices throughout the study, as well as significant decreases in body weight gain in mice from 52 weeks onward. For this reason, the highest dose group was excluded from the analysis.

Cancer dose-response data are also available from studies in F344 rats (DeAngelo et al., 1996; Richmond et al., 1995). This data set is less robust than that for mice, having only three dose groups, the highest of which apparently exceeded the MTD, based on a marked decrease in body weight. In addition, the data were collected in two separate studies in two separate laboratories. Therefore, these data were not used in the cancer benchmark dose modeling.

5.3.2. Dose-Response Data

Table 5-8 summarizes the tumor incidence data for liver carcinomas in $B6C3F_1$ mice in the study by DeAngelo et al. (1999). The numbers of animals with either adenomas or carcinomas at 100 weeks were used to model the cancer dose-response relationships.

Table 5-8. Cancer dose-response data evaluated using BMD modeling: male mice[a]

Conc. in water (g/L)	No. of animals entering study	Mean BW (g) at 100 weeks	Dose (mg/kg-day)	Animals with hepato-carcinomas at 100 weeks	Animals with hepato-adenomas at 100 weeks	Animals with either hepato-carcinomas or adenomas at 100 weeks

			Animal	HED [b]	%	N	%	N	%	N
0	50	43.9	0	0	26%	13	10%	5	36	18
0.05	33	43.3	8.0	1.3	33%	11	3%	1	33	11
0.5	25	42.1	84	13.2	48%	12	20%	5	56	14
1	35	43.6	168	26.5	71%	25	51%	18	86	30
2	21	36.1	315	47.5	95%	20	43%	9	100	21
3.5	11	36.0	429	64.6	100%	11	45%	5	100	11

(a) High-dose group excluded from benchmark modeling (see text)
(b) HED calculated using a dose scaling factor of $BW^{0.75}$
Source: DeAngelo et al. (1999)

5.3.3. Dose Conversion

Because the exposure of mice to DCA in drinking water was continuous for the approximate full life span of the animals, no adjustment is needed to account for duration of exposure or duration of study. Doses in animals are converted to human equivalent doses (HED) by assuming that doses (mg/day) in animals and humans are toxicologically equivalent when scaled by body weight raised to the 3/4 power (U.S. EPA, 1992):

$$\left(\frac{\text{Dose (mg/day)}}{BW^{3/4}}\right)_{\text{animal}} = \left(\frac{\text{Dose (mg/day)}}{BW^{3/4}}\right)_{\text{human}}$$

When doses are expressed as mg/kg-day, this yields the following:

$$\text{HED (mg/kg-day)} = \text{Dose in animals (mg/kg-day)} \cdot (BW_a / BW_h)^{0.25}$$

The group mean body weights for animals in each exposure group are shown in Table 5-8 above. The body weight of humans was assumed to be 70 kg (U.S. EPA, 1988). The resulting HED values are also shown in Table 5-8.

5.3.4. Dose-Response Characterization in the Range of Observation

The dose-response data sets presented in Table 5-8 were modeled using the BMDS software system (Version 1.3.1) developed by the U.S. EPA National Center for Environmental Assessment (NCEA). The benchmark dose was estimated using the numbers of animals with

adenomas or carcinomas in the five lowest dose groups. As noted above, the highest dose group was excluded because the highest dose (429 mg/kg-day) approached the MTD. The results of the model fitting are detailed in Appendix D, and the findings are summarized in Table 5-9.

Judging by the chi-squared *p*-values, all the models except the quantal linear fit the data reasonably well (*p*-values > 0.7). The multistage and quantal quadratic models had the highest *p*-values (0.981) and the lowest AIC values (174.62), and thus appear to provide the best fit to the data. For a benchmark risk (BMR) level of 0.10, the estimated benchmark dose values for the best-fitting models (*p*-values > 0.7) range from 3.1 to 9.4 mg/kg-day, and the BMDL values range from 2.1 to 5.7 mg/kg-day.

5.3.5. Selection of a Dose-Response Model

The multistage and quantal quadratic models provide essentially identical fits to the data. The multistage model estimate was selected for dose-response extrapolation because the quantal quadratic model has no first-order term and therefore may predict zero slope at zero dose. Given the uncertainty surrounding the carcinogenic mechanism of DCA (see Section 4.6), it was decided that the zero slope assumption was not justified. The multistage model gives a BMDL estimate of 2.1 mg/kg-day (2.05, rounded to two significant figures). The fit of the multistage model to the DeAngelo et al. (1999) data is shown in Figure 5-2.

Table 5-9. Summary of cancer BMD modeling results[a,b]

Model	BMD	BMDL	p-value	AIC
Multi-Stage (2)	6.86	2.05	0.981	174.62
Quantal-quadr.	6.86	5.69	0.981	174.62
Probit	3.16	2.54	0.816	175.54
Logistic	3.10	2.43	0.728	176.11
Weibull	7.53	2.50	0.916	176.59
Gamma	8.45	2.55	0.858	176.81
Log-Probit	9.36	4.27	0.779	177.10
Log-Logistic	9.17	4.07	0.703	177.45
Quantal-linear	1.88	1.37	0.370	178.41

(a) Data = DeAngelo et al. (1999), animals with hepatocarcinoma or adenoma, excluding high-dose group
(b) BMD estimated using BMR = 0.10, BMDL estimated as 95% LCL

5.3.6. Extrapolation to Doses Below the Range of Observation

Selection of the Point of Departure

Based on the data summarized above, the point of departure (POD) selected for the quantification of cancer risk from DCA is the BMDL of 2.1 mg/kg-day, derived from the fit of the multistage model to the cancer incidence data in male mice, with the high-dose group excluded.

Extrapolation to Low Dose

In 1996, the U.S. EPA published its Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a). The guidelines were recently updated by the Agency (U.S. EPA, 1999, 2003). Under the revised guidelines, two alternative approaches may be used to quantify cancer risk, depending on what is known about the mode of carcinogenicity and the shape of the dose-response curve. A linear default approach is used for a chemical when available evidence indicates that the chemical is mutagenic or DNA-reactive, or supports another mode of action that is anticipated to be linear. An inference of linearity may also be supported if existing human exposure is thought to be on the linear part of a dose-response curve, even though the overall dose-response is sub-linear. The linear approach is used as a matter of policy if the mode of carcinogenicity is not understood. Non-linear models may be used when the mode of carcinogenicity is reasonably understood, and the weight of evidence supports the conclusion that the dose-response curve is likely to be nonlinear.

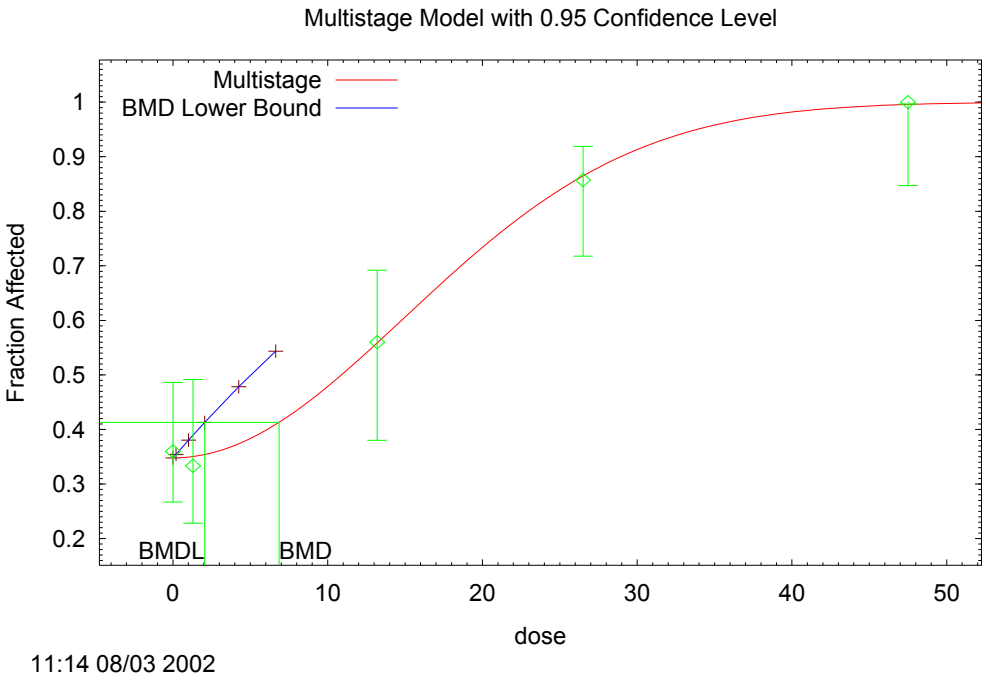

Figure 5-2. Multistage dose-response model fit for combined hepatocarcinoma and adenoma incidence in male mice

As discussed in Section 4.6, in the case of DCA, available data are not sufficient to establish a cancer mode of action with reasonable certainty, especially at the very low exposure levels expected to apply to humans ingesting chlorinated drinking water. Therefore, in accord with EPA's Proposed and Draft Final Cancer Assessment Guidelines (U.S. EPA, 1999, 2003), extrapolation from the POD to low dose is performed by assuming a linear dose-response curve between the POD and the origin. The slope factor derived from the central tendency estimate of the cancer response is simply the BMR divided by the BMD.

$$\text{Slope Factor} = \text{BMR} / \text{BMD} = 0.1 / (6.9 \text{ mg/kg-day}) = 0.015 \text{ (mg/kg-day)}^{-1}$$

The slope factor derived from the BMDL is calculated as follows:

$$\text{Slope factor} = \text{BMR} / \text{BMDL}_{10} = 0.1 / (2.1 \text{ mg/kg-day}) = 0.048 \text{ (mg/kg-day)}^{-1}$$

5.3.7. Confidence in the Cancer Assessment

Available data are adequate to establish with high confidence that high doses of DCA cause liver cancer in mice and rats. Because the mode of cancer induction by DCA is not understood, there is considerable uncertainty as to whether low doses of DCA (as ingested by

humans who consume chlorinated drinking water) are also likely to increase cancer risk. Assuming that low doses of DCA are carcinogenic, then the estimate of cancer potency derived from the dose-response study in male mice is considered to be reliable.

The BMDL is estimated based on four dose groups plus a control, from a high-quality study. The BMDL estimate is moderately sensitive to the model selected to estimate it, but the best-fitting models, which do not assume zero slope at zero dose, all predict BMDLs that are approximately within a factor of two of the multistage model, with the majority of models predicting BMDLs between 2.0 and 3.0 mg/kg-day.

Based on these considerations (strong dose-response data and good model fits in mice, but lack of understanding of the mode of action), confidence in the quantification of the cancer risk for DCA is rated as *medium*.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE-RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Exposure Pathways

Dichloroacetic acid occurs at low levels in most drinking water systems that are disinfected with chlorine. Humans are exposed mainly by ingestion of chlorinated drinking water. Dermal contact may also occur during showering or bathing. Because DCA has low volatility, inhalation exposure is not expected to be significant.

Toxicokinetics

Dichloroacetic acid is well absorbed by the gastrointestinal tract and is distributed to multiple tissues throughout the body. Dichloroacetic acid is metabolized in the liver by oxidative dechlorination to yield glyoxylate, which can enter intermediary metabolism and either be oxidized to oxalate and excreted, converted to carbon dioxide, and/or incorporated into amino acids or other cellular molecules. In most species, including humans, clearance of DCA from the plasma is relatively rapid, with a half-time of 2-3 hours for single doses. In contrast, limited data suggest that dogs clear single doses of DCA more slowly. Repeated exposure to DCA results in a decreased ability to metabolize DCA in humans and animals, most likely because DCA inhibits GSTZ, the cytosolic enzyme needed to carry out the metabolism of the parent compound via oxidative dechlorination. Inhibition of DCA metabolism can result in blood levels of DCA that are 8 to 10 times higher than after single doses. DCA can also be metabolized to monochloroacetic acid and thiodiacetic acid.

Characteristic Non-cancer Effects

Dichloroacetic acid causes a wide spectrum of adverse effects in animals and humans, including:

Effects on Metabolism

Dichloroacetic acid treatment results in a significant reduction in plasma levels of glucose, pyruvate and lactate. This finding has been consistently reported in DCA-treated rats, dogs, and humans. Metabolic effects are seen in humans at oral doses as low as 10 mg/kg-day. DCA exposure also decreases plasma cholesterol levels. The metabolic effects of DCA have led to its experimental use in the treatment of lactic acidosis, diabetes, and familial hypercholesteremia. As noted in the section on hepatotoxicity below, DCA exposure induces a marked accumulation of glycogen in the liver and thus bears some similarity to glycogen storage disease VI in humans. The metabolic basis for this accumulation has not been elucidated. Most DCA-induced metabolic alterations appear to be transient, with full recovery to basal/control levels following cessation of DCA administration, although in at least one study, liver glycogen levels in mice became resistant to change after 8-weeks of DCA administration (Kato-Weinstein et al., 1998).

Hepatic Toxicity

DCA causes a dose-related increase in liver size, generally accompanied (or caused) by an increase in glycogen deposition in the liver. Liver toxicity, as evidenced by increases in serum levels of liver enzymes, has been seen in DCA-treated mice, rats, dogs, and humans. Hepatic necrosis has been consistently reported in mice exposed to high doses of DCA. Frank liver necrosis has not been seen in rats, even at the highest concentration used, nor has it been reported in dogs or humans.

Reproductive/Developmental Toxicity

In males, DCA causes decreases in testicular weight and viable sperm production. Testicular effects were observed in rats and dogs. Dogs are apparently the most sensitive species, displaying testicular toxicity at a dose substantially lower than for other test species. In

female rats, DCA exposure during gestation can lead to impaired fetal maturation and result in soft tissue anomalies (primarily of cardiac origin) in the offspring.

Neurotoxicity

In humans, exposure to DCA causes sedation in many individuals, and occasionally results in reversible peripheral neuropathy. Neurological effects have also been reported in rats and dogs, including hind limb paralysis and morphologic alterations in the CNS. Gait abnormalities have been observed in weanling and adult rats exposed to DCA either by gavage or via the drinking water route. To date, signs of neurologic toxicity or morphologic changes of the nervous system in DCA-treated mice have not been reported.

Characteristic Cancer Effects

There are no apparent studies which have been conducted to explore whether DCA exposure is associated with increased cancer risk in humans. Multiple studies in rodents, however, have revealed that DCA exposure causes increased incidence and multiplicity of hepatic adenomas and adenocarcinomas. This effect has been observed in male and female mice and in male rats. Livers of DCA-treated mice displayed adenomas and carcinomas that developed from eosinophilic, dysplastic, basophilic or clear cells indicating several origins for the tumorous growths. Increased tumors have not been observed in other rodent tissues.

Mode of Action

Non-cancer Effects

Dichloroacetic acid is known to inhibit the protein kinase that maintains pyruvate dehydrogenase in its inactive form. By inhibiting this protein kinase, the activity of pyruvate dehydrogenase is increased, which in turn results in a spectrum of changes in intermediary metabolism, including a decrease in plasma glucose and lactate. Dichloroacetic acid has also been shown to be a noncompetitive inhibitor of the rate limiting microsomal enzyme in cholesterol biosynthesis (HMG CoA reductase), which likely accounts for its effect on plasma cholesterol levels. It may inhibit glycogen phosphorylase b kinase or a debranching enzyme leading to hepatic glycogen accumulation and GSTZ, possibly leading to the accumulation of alkylating tyrosine metabolites.

The detailed mode of DCA-induced hepatotoxicity is not known. Potential modes that have been suggested include peroxidative damage secondary to DCA metabolism, abnormal glycogen storage, infarction caused by extensive liver hypertrophy, and disruption of cell cycle control through DNA/RNA-centered changes related to signal transduction and nuclear transcription factors. No specific cellular or molecular hypotheses have been advanced to explain the neurotoxicity and reproductive toxicity of DCA, but inhibition of key cellular enzymes in the affected tissues is likely to be involved. The potential relevance of GSTZ inhibition to the toxic mechanism of DCA is not known.

Cancer Effects

A number of potential modes of DCA-induced hepatocarcinogenicity have been proposed, including the following:

Direct-acting Genotoxicity

The genotoxicity data base on DCA has been extensively reviewed by several scientific organizations including IARC (1995), ILSI (1997), WHO (2000), and U.S. EPA (1998c). Based on an evaluation of data available at the time, IARC (1995) and ILSI (1997) each reached independent conclusions that DCA was not genotoxic. More recently, WHO (2000) concluded that, although there is some evidence of DCA being genotoxic, these effects occur at such high concentrations that they are not likely to be involved in the mode of DCA tumorigenesis. In contrast, NCEA (U.S. EPA, 1998c) concluded that available data indicate that DCA is a direct acting genotoxic agent. NCEA (U.S. EPA, 1998c) considered new data that were not available to IARC and ILSI during their respective reviews. NCEA (U.S. EPA, 1998c) stated that the test results reveal the ability of DCA to cause mutational damage, both point mutations and chromosomal aberrations, although generally at relatively high exposure levels. NCEA (U.S. EPA, 1998c) also indicated that mutations are viewed as exhibiting linear low-dose responses according to EPA's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2003). Because the data on DCA genotoxicity *in vivo* are mixed, and because no clear explanation for the internal disagreement between studies is apparent, EPA considers it prudent to assume that DCA might be genotoxic, at least under *in vivo* exposure levels that are associated with detectable increases in tumor incidence. Whether DCA is genotoxic at lower doses is not known.

Hepatocytotoxicity and Regenerative Hyperplasia

Dichloroacetic acid causes focal or widespread hepatic necrosis in mice at high doses, and regenerative hyperplasia occurs in some animals in which a tumorigenic response is observed. However, hepatotoxicity and regenerative hyperplasia have not been observed in rats. In addition, hepatotoxicity and regenerative hyperplasia have not been observed in mice exposed to low doses of DCA that were associated with tumor formation. For instance, DeAngelo et al. (1999) reported an increased cumulative incidence and multiplicity of hepatic tumors in male mice that exhibited no apparent cytotoxic or regenerative response to DCA. Further, treatment of rodents with DCA for longer than two weeks decreases cell replication rates (Stauber and Bull, 1997), indicating that any regenerative hyperplasia occurring shortly after initiation of treatment is not sustained. The data indicate that this mode of action is not likely to play a role in DCA-induced hepatocarcinogenicity.

Promotion of Spontaneous Mutations

There are several sets of observations suggesting that DCA may be acting through a tumor promotion mechanism, including inhibition of proliferation of normal hepatocytes and stimulated proliferation of c-*Jun* positive hepatocytes *in vitro,* and increased hepatic tumor multiplicity in mice exposed to DCA followed by phenobarbital. The majority of cancer bioassays, however, indicate that DCA is a complete carcinogen, because it is capable of inducing cancer when administered alone, both at high doses in short-term assays (50-60 weeks), and at lower doses with longer exposure periods ($\geq$100 weeks).

Depression of Apoptosis

A proposed general mechanism of tumor promoters is the decreased apoptosis of initiated cells in a tissue by a promoting compound, thereby allowing the outgrowth of cells previously programmed to die. Snyder et al. (1995) showed that DCA decreased spontaneous apoptosis in liver cells of mice exposed to DCA (0.5 or 5 g/L) for up to 30 days. Further, Stauber and Bull (1997) determined that DCA treatment induced primarily small eosinophilic lesions or foci (1-100 cells), an observation confirmed by Miller et al. (2000) in their study of DCA-induced tumor growth. A proposed mechanism of carcinogenesis is that DCA is causing the formation of these small lesions through suppression of apoptosis.

In summary, available data are not adequate to indicate which mode(s) of action is responsible for the hepatic tumor response of rats and mice to DCA exposure.

6.2. DOSE-RESPONSE

Oral RfD

Available data suggest that all of the characteristic noncancer effects in humans and animals occur with similar dose-response patterns, with effect levels of about 25 mg/kg-day or higher. However, the dog appears to be somewhat more susceptible, at least with respect to testicular effects in the male. In this case, clear effects have been noted at a dose of 12.5 mg/kg-day (Cicmanec et al., 1991), while no NOAEL has been established. Besides testicular toxicity, neurological changes, hepatic vacuolization, and increased liver weight were observed in males and females at 12.5 mg/kg-day. The basis for the increased sensitivity of the dog to testicular toxicity is not certain, but it may be due to relatively low capacity to metabolize DCA and clear it from the plasma. However, since nothing is known about the mechanism of the testicular toxicity and the metabolism of DCA is complex, especially after repeated dosing, there is no basis for concluding that metabolism alone is responsible for testicular effects in dogs at low doses. Using the LOAEL of 12.5 mg/kg-day identified in the dog, and applying an uncertainty factor of 3000 to account for use of a LOAEL, extrapolation from animals to humans, and potential inter-human variability in sensitivity, a duration adjustment and database deficiencies, an oral RfD of 4.2E-03 mg/kg-day has been derived. Data from study by Cicmanec et al. (1991) could not be reliably evaluated using the BMD approach.

The overall confidence in the RfD is medium. No adverse effects have been reported in humans at doses lower than 25 mg/kg-day, but systematic investigations of potential hepatic or reproductive effects in therapeutically-treated humans have not been performed. Metabolic effects are seen in humans at oral doses as low as 10 mg/kg-day. Limited toxicokinetic data indicate that humans clear single DCA doses from the plasma more rapidly than dogs, but data on comparative metabolism in humans and dogs after multiple doses are lacking, and it has yet to be established whether the various aspects of DCA toxicity are due to the parent compound or one or more metabolites.

Oral Cancer Risk

The cancer risk from ingestion of DCA was quantified based on a dose-response study in male mice (DeAngelo et al., 1999). The cumulative incidence of hepatic total tumor incidence (carcinoma plus adenoma) in the test animals was well-described by several different dichotomous models, with the multistage model yielding the best fit. Based on this model, the BMD (the dose that caused a 10% increase in extra risk) was 6.86 mg/kg-day, and the BMDL was 2.1 mg/kg-day. In accordance with guidelines (U.S. EPA, 1999), the BMDL was used as the point-of-departure (POD) for quantifying cancer risk. Because the mode of action by which DCA increases cancer risk is not understood, extrapolation to low dose was performed by assuming a no-threshold linear dose-response curve between the origin and the POD. This yields a cancer slope factor of 0.048 $(mg/kg\text{-}day)^{-1}$.

Dichloroacetic acid is a *likely human carcinogen* that lacks a cohesive mode of action. This assessment is based on the strength of the evidence in several animal bioassays and is supported by mechanistic data that suggest a complex etiology for tumor development. There are a number of independent studies reporting consistently positive carcinogenic results at roughly comparable doses, site concordance for tumor formation between two species, consistent observations in different species and sexes, and clear evidence of a dose-response relationship. The data on mechanism implicate more than one type of cellular change in the origin of tumors along with defects in intra- and inter-cellular communication pathways. Accordingly, the use of a linear extrapolation of dose is appropriate in quantifying the cancer risk for DCA.

Inhalation RfC and Cancer Risk

There are no studies of inhalation exposure to DCA. DCA has low volatility, and inhalation exposure to DCA is not believed to be a significant exposure pathway for most people. Therefore, no inhalation RfC or unit risk value have been derived.

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APPENDIX A

RESPONSE TO PEER REVIEW SUMMARY DOCUMENT

APPENDIX A

External Peer Review—Summary of Comments and Disposition

The draft Toxicological Review and the IRIS Summary for Dichloroacetic Acid have undergone internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1994d). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows.

Disposition of Specific Charge Questions

Question 1. Does the documentation successfully communicate the essential components and findings of the source documents?

Comment: One reviewer thought that the examination of the database across studies and discussions of modes of action should have involved a more substantial and critical discussion, making use of other information from the basic sciences and the toxicology of other chemicals.

Response: Revisions to the report have focused on increasing the background information for a number of the mechanistic studies in Section 4.4.1. and providing more integration of data in the synthesis sections (Sections 4.5. and 4.6.) of the Toxicological Review.

Comment: One reviewer suggested that there be more discussion regarding inconsistencies in results across similar studies.

Response: Additional discussion regarding inconsistencies across similar studies, particularly in regards to the genotoxicity studies in Section 4.4.2, has been added.

Comment: One reviewer was concerned that the external review draft appeared to deviate from the Agency's cancer guidelines and other precedents by establishing the dual hazard classification in the case where the dose-response relationship is uncertain ("likely to be a carcinogen at high

exposure levels, but cannot be classified at exposure levels that are associated with environmentally relevant exposure conditions"). The reviewer indicated that this was inconsistent with the Agency's March 1998 Carcinogenicity Identification Characterization Summary and recommended that this change be reviewed.

Response: The cancer classification has been changed and is no longer a "dual classification."

Question 2. Are there any significant publications that are not included in the Toxicological Review document?

Comment: One reviewer noted several papers related to the metabolism of DCA that seem to have been overlooked, including:

Austin, EW and Bull, RJ (1997) Effect of pretreatment with dichloroacetate or trichloroacetate on the metabolism of bromodichloroacetate. J Toxicol Environ Health 52:367-383.

Austin, EW; Parrish, JM; Kinder, DH; et al. (1996) Lipid peroxidation and formation of 8-hydroxyguanosine from acute doses of halogenated acetic acids. Fundam Appl Pharmacol 31:77-82.

Lingohr, MK; Thrall, BD; Bull, RJ. (2001) Effects of dichloroacetate (DCA) on serum insulin levels and insulin-controlled signaling proteins in livers of male B6C3F1 mice. Tox Sci 59:178-184.

Schultz, IR; Merdink, JL; Gonzalez-Leon, A; et al. (1999) Comparative toxicokinetics of chlorinated and brominated haloacetates in F344 rats. Toxicol Appl Pharmacol 158:103-114.

Xu, X; Stevens, DK; Bull, RJ. (1995) Metabolism of bromodichloroacetate in B6C3F1 mice. Drug Metab and Disp 23:1412-1416

The reviewer suggested that EPA conduct a more expansive literature search to include the technically more correct terms: dichloroacetate, haloacetates, and haloacetic acids.

Response: The suggested papers were obtained and reviewed. In addition, a literature search was conducted covering the period from the original literature search (1998) to the present. Papers identified from that search were also retrieved and reviewed. Some, but not all of the papers suggested by the reviewer and identified in the new literature search were added to the Toxicology Review. Studies that were not assimilated (Austin and Bull [1997]and Lingohr et al. [2001]) were not added to the report because they did not significantly augment the DCA

discussions in the revised report.

Comment: One reviewer recommended including the following study in the Toxicological Review:

Spruijt, L; Naviaux, RK; McGowan, KA; et al. (2001) Nerve conduction changes in patients with mitochondrial diseases treated with dichloroacetate. Muscle Nerve 24(7):916-924.

The reviewer also commented that the study should be considered for inclusion in Table 5-1, Figure 5-1, and for comparison in the reference-dose calculations.

Response: Data from the paper was included in the section for noncarcinogenic, systemic effects. The data were also included in Table 5-1 and in Figure 5-1.

Comment: One reviewer recommended that the following recent papers on DCA mechanism of action be included:

Pereira, MA; Kramer, PM; Conran, PB; et al. (2001) Effect of chloroform on dichloroacetic acid and trichloroacetic acid-induced hypomethylation and expression of the *c-myc* gene and on their promotion of liver and kidney tumors in mice. Carcinogenesis 22(9):1511-9

Thai, SF; Allen, JW; DeAngelo, AB; et al. (2001) Detection of early gene expression changes by differential display in the livers of mice exposed to dichloroacetic acid. Carcinogenesis 22(8):1317-22.

Response: The studies have been included in the document.

Comment: One reviewer wanted additional discussion of the potential for DCA to mediate the carcinogenesis of other chlorinated compounds (e.g., per- and trichloroethylene) because data show that these compounds are metabolized into DCA *in vivo* (see the IARC Monograph [IARC, 1995] on perchloroethylene for discussion).

Response: The draft external peer review document discusses briefly the relevance of metabolism of perchloroethylene and trichloroethylene to DCA and resultant carcinogenesis. A significant amount of text has not been devoted to this subject, because some studies indicate that the amount of DCA produced from trichloroethylene exposure (and possibly tetrachloroethylene) is small (acute exposure to a large dose; Brüning et al., 1998) and within the amount that would be readily metabolized by GSTZ (Barton et al., 1999). The EPA is currently reevaluating these chemicals as

part of the IRIS file, the metabolism of these compounds to DCA will be discussed in those documents when completed.

Question 3. Are there any problems with the quantification of the DeAngelo et al. (1999) data and the use of that data to quantify the carcinogenicity of DCA?

Comment: One reviewer noted that the DeAngelo et al. (1999) data are probably the most appropriate as a basis for modeling cancer risk, but the Pereira et al. (1996) data should have been considered because it used female mice whereas the DeAngelo et al. (1999) only used male mice. The reviewer indicated that although other studies in male mice have fewer doses, it would still be useful to point out that there is general agreement across many studies identifying the amount of DCA that is required to consistently induce cancer in male mice. Therefore, the reviewer feels that the DeAngelo study is a surrogate for a larger data base.

Response: The DeAngelo et al. (1999) study was chosen because it represented a longer duration and because a comparison between the two studies of tumor multiplicity and the development of carcinomas indicate that female mice are less susceptible to the carcinogenic effects of DCA than are the males (e.g., they do not develop as many tumors or as many carcinomas as males at comparable doses).

Comment: One reviewer noted that the wrong data sets were used in the benchmark dose modeling effort for the cancer endpoint, and the correct data sets did not appear in the Toxicological Review for either the rat or mouse experiment. The reviewer stated that the main data set for each species would be the number of animals with tumors: (1) at the site of interest, (2) of the same embryonic origin, and (3) that are malignant or have the potential to become malignant. In this particular case, any animal with a hepatocellular adenoma or carcinoma would be counted.

Response: An error in the draft report was corrected and the dose-response was modeled on the number of animals with hepatocellular adenomas or carcinomas.

Question 4. The toxicological papers have been arranged in reverse chronological order within specific sections. Does this cause any problem to you as a reader of the document?

Comment: One reviewer indicated that the format did not make it easy to discern how

information has developed on the toxicodynamics of DCA's effects and that the document occasionally indicate that a result was confirmed in the first paper put out on the subject.

Response: EPA has changed the order of the articles in the section to reflect groupings by effect and by animal model. The change is intended to help facilitate comments that compare and contrast results from similar studies, and better describe changes in thinking regarding mode of action. The format of presentation has also been altered so that the discussions are more integrated than they were with the study-by-study approach.

Comment: One reviewer preferred the reverse chronological order in which the toxicity studies were reviewed since it allowed the reader to consider the most recent citations first. In this reviewer's opinion, more recent studies tend to be of higher quality, particularly from the standpoint of more standardized and complete protocols, and should be the primary focus.

Response: Although EPA has revised the format for reporting the studies, an attempt has been made to insure that the data are presented in a cohesive, reader-friendly order.

Comment: One reviewer found the format to be distracting of the study-by-study descriptions under Sections 4.1. to 4.4.

Response: It is difficult to synthesize the results of so many studies without losing details regarding methodology. In EPA's revisions to the document, the details of the individual studies have been maintained even though the format has been altered.

Question 5. Do you have any technical disagreement with the information presented?

Comment: One reviewer thought that the external review draft did not adequately address the modes of action and that some hypotheses were maintained in the document that were no longer supported in the scientific literature. Further discussion of alternate explanations, such as distribution toward small lesions, potentially due to suppression of apoptosis, should be added.

Response: EPA has made extensive revision to the External Review Draft of the DCA Toxicological Profile. Some of these revisions were initiated as a response to the reviewer's comments. The discussions of the mechanistic and genotoxicity studies have been expanded and new data regarding precursor lesions found in the liver have been added to the Toxicological

Review.

Comment: One reviewer indicated that some genotoxicity sections implied a greater degree of uncertainty than was warranted regarding whether DCA should be considered genotoxic. This reviewer found the genotoxicity data on DCA neither convincing nor supportive of a weight-of-evidence based conclusion that DCA is mutagenic. The reviewer believed that the mode of action for DCA should still be considered uncertain, despite the broad ranging investigations.

Response: EPA has rewritten the genotoxicity section to reflect the preponderance of evidence that DCA is nongenotoxic, except at high doses, in the models assessed.

Comment: One reviewer objected to the term "prevalence" used in the document and recommended text changes to replace the term.

Response: The recommended changes have been made.

Comment: One reviewer noted that the ancillary statistics on the occurrence of adenomas and carcinomas in the same animal could be removed from the tables and strongly suggested that the next revision of the document be reviewed by experts at the National Center for Environmental Assessment (NCEA).

Response: The data have been removed. The document has been reviewed by NCEA and other individuals familiar with benchmark dose methodology. No specific critical comments regarding the approach were raised.

Comment: One reviewer questioned a statement in the original draft regarding the absence of reductive dechlorination in humans; the statement was made in the draft based on the apparent lack of evidence of thiodiacetic acid or monochloroacetic acid excretion in humans. The reviewer indicated that the basis for the statement was a set of clinical observations on adults with diabetes by a research group out of the University of Florida at Gainesville. Further, the reviewer felt that the reader should be aware that such conclusions were based on clinical observations in adults in a specialized population exposed to a variety of other medications. The issue of heterogeneity and the potential for a subpopulation for which this pathway might be important should be considered.

Response: The entire pharmacokinetic section has been rewritten and there is no longer mention of reductive chlorination not being a relevant metabolic pathway in humans. There are human data indicating that there was excretion of monochloroacetic acid in one human subject. Accordingly, the original text was incorrect.

Comment: One reviewer indicated that the original draft review document gave the reader the impression that multiple doses of DCA were metabolized similarly to one dose. The reviewer indicated that studies currently suggest that humans may not be able to metabolize multiple doses of DCA readily and suggested that the report should not focus so much on single-dose studies when comparing differences between species in the ability to metabolize DCA.

Response: The entire pharmacokinetic section has been rewritten. The document now includes considerable discussion on the results of high doses and pretreatment on DCA metabolism including discussion of half-life.

Comment: With regard to the discussion concerning the change in the elimination half-life with multiple dosing, one reviewer indicated that the 1991 paper by Curry should be discussed in the paragraph on page 5, lines 24-34.

Response: The comment has been addressed in the revisions to the toxicokinetic section of the Toxicological Review.

Comment: One reviewer suggested that the Toxicological Review notes that the human studies with DCA were performed on individuals who were therapeutically treated with DCA, and thus were an unhealthy population. No analytic epidemiological studies have been performed to date, nor have any of the studies had sufficient power to detect carcinogenicity. Clear statements notifying the reader to that effect should be added.

Response: The recommended changes have been made.

Comments: One reviewer provided the following comments regarding the selection criteria for the application of benchmark dose modeling to noncancer data sets: (1) a reference for the criteria provided on page 57 would be useful for the reader and (2) the exclusion of effects which are reversible can be questioned, especially in cases where the environmental exposures may last a lifetime, and especially in cases where the effects are as severe as observed in the study in

question [on page 49, at lines 24-25]. The reviewer also indicated that the nature and severity of the endpoint and the length of exposure should be carefully considered in such an exercise.

Response: The recommended change for point (1) above has been made. EPA has changed the text regarding the neurotoxicity effects in rats as suggested by point (2) and removed the "slow reversibility" comment. It is maintained that the study should not be used for the development of a RfD since it did not identify a NOAEL and the lowest LOAEL was higher than that identified for testicular effects in the Cicmanec et al. (1991) study.

Comments: One reviewer provided the following comments with regard to the presentation of data on genotoxicity:

- Table 4-2 indicated all mutagenicity studies by Herbert et al. (1980) are negative or equivocal. Yet, in this reviewer's opinion, the authors concluded that "Dichloroacetate demonstrates low grade mutagenicity in the Ames Salmonella/mammalian microsome mutagenicity test."

- Table 4-2 indicated the findings from DeMarini et al. (1994) in the microscreen prophage-induction assay as +/-, but the published study clearly states that DCA was genotoxic in that assay.

- Table 4-3 did not present multiple positive results from the Fuscoe et al. (1996) study, excluding a small but statistically significant increase in the frequency of micronucleated normochromatic erythrocytes after 10 weeks of exposure, and a positive finding by alkaline single cell gel electrophoresis indicating cross-linking in addition to the frequency of micronucleated polychromatic erythrocytes. The reviewer suggested that the text indicate that these authors also coadministered vitamin E and found it did not affect DNA damage induction by dichloroacetic acid.

- The Ono et al. (1991) and Waskell et al. (1978) studies on DNA repair should be included in Table 4-2.

- It should be noted in the Toxicological Review that Harrington-Brock et al. (1998) found the potency of DCA similar to the classic mutagen ethylmethanesulfonate.

- The genotoxicity tables should indicate that Chang et al. (1992) found that 5 and 10 mmole/kg DCA produced a small amount of strand breakage in mice (7% at 4 hr). Strand breaks from continuous exposure to DCA were also elevated slightly in the mouse. Table 4-3 should indicate that the splenocytes and epithelial cells in the Chang et al. (1992) study were derived from the stomach and duodenum.

- The reviewer suggested an interpretation of the transgenic mice data indicating that a

direct genotoxic effect would be time independent and that since the findings were not observed at 4 and 10 weeks but were at 60 weeks, the mutational events might be secondary to toxicological changes in the liver. The reviewer said this interpretation did not take into account the greater DCA exposure resulting from the prolonged exposure.

- The original draft indicated that DCA has been consistently negative in all standard DNA cross-linking studies conducted and provided a secondary reference as justification. The reviewer indicated that the primary references should be cited.

- The analysis of data regarding mutagenicity involving study counts of numbers of positives and negatives was superficial (page 30). The reviewer indicated that a more analytic presentation, providing the context for the results in terms of endpoints examined, and power of studies to detect weak effects, was needed.

Response: Changes to the text on genotoxicity have been made to present a more accurate presentation of positive and negative results, as well as to compare and contrast findings from similar studies.

Question 6. Do you agree with the selection of the critical study and effect or the assignment of uncertainty factors for determination of the RfD?

Comment: One reviewer agreed with the choice of the Cicmanec et al. (1991) study as the principal study and the critical endpoint but was uncomfortable with the justification for not modeling the non-monotonic data using benchmark dose (BMD) response. The issue is where to select the point of departure in such a case, and this should be stated in the document. In this reviewer's opinion, a better argument for not performing a BMD analysis is that the endpoint is trivial and not interpretable in a health sense (e.g., weight gain at low doses of DCA in DeAngelo et al., [1996]). The reviewer indicated that RfD values developed by a BMD analysis and the NOAEL/LOAEL approach would be comparable because different uncertainty factors could be used.

Response: EPA has modified the text to reflect the recommended comment regarding point of departure for non-monotonic data modeled using BMD techniques. EPA has not added the changes regarding the comparability of RfDs developed by a BMD analysis and more traditional NOAEL/LOAEL approach because the changes would complicate this section of the document. Revisions to the discussions on the derivation of the total uncertainty factor have been made, however.

Comment: One reviewer indicated that is was appropriate to use the Cicmanec et al. study (1991) as the critical study and testicular degeneration as the critical effect for calculating the RfD. Further, the basis for using a NOAEL/LOAEL approach rather than the BMD was well-documented and explained. The reviewer believed the factor of 10 for the interspecies extrapolation could be reduced based on an assessment of the metabolic differences between the dog and humans, indicating that the dog may be more sensitive than humans, rather than the opposite. The reviewer also indicated that it appears to be appropriate to use the DeAngelo et al. (1999) data for determining the dose-response for the cancer assessment.

Response: The uncertainty factor for the extrapolation from human to animals based on the metabolic data on the effects of preexposure on metabolism in humans and animals has been reduced. The data on similarities between humans and animals with regard to DCA metabolism justify a decrease in the interspecies uncertainty factor to 3 rather than 10. However, on close examination of the metabolic data in dogs, EPA now feels they are not particularly strong. EPA added a 3-fold factor for data base uncertainty because of the lack of a multigeneration study of DCA. Accordingly, the total uncertainty factor remains at 3,000.

Comment: One reviewer thought the derivation of the noncancer RfD was clearly laid out and specific calculations were well justified. Further, the reasoning leading to the exclusion of the benchmark dose calculations from selection of the RfD was also appropriate. The reviewer had reservations regarding the selection of uncertainty factors and suggested that the potential exists for wide human variability (e.g., Curry et al., [1991] and the GSTZ polymorphism and other factors noted in the Toxicological Review). Therefore, these findings, which are based on limited observations, increase the concern (the small number of individuals studied and the limited types of observations made biases the investigation toward a false negative). Further, the LOAEL was identified at a high response – 80% of the dogs in the critical study were found with testicular degeneration and suggested that a factor of 10 may not be sufficient to predict from an 80% incidence what might be observed as a NOAEL in a study of reasonable size (e.g., with 50 animals per dose group).

Response: The 10-fold uncertainty factor for intraspecies variability has been retained. The discussion on why this factor was selected and the discussion on sensitive populations have been expanded to provide greater support for this decision. The UF of 10 for the use of a LOAEL was also retained, however a threefold uncertainty factor was added for data base uncertainty due to the lack of a multigeneration study of reproductive toxicity which would include consideration of

the functional impact of the testicular effects on reproduction.

Question 7. Do you have any suggestions for improving the presentation of information that are compatible with IRIS SOPs?

Comment: One reviewer believed that the mode of action section did not include enough critical examination of conflicting data in available studies. Further, additional efforts should be included regarding how the mode of action data have been developed for DCA.

Response: The text has been modified to improve the handling of mode of action data.

Comment: One reviewer suggested the following changes: (1) reformat the tumor incidence tables; (2) include a schematic of hypothesized metabolism of DCA; (3) provide introductory paragraphs before each major section orienting the reader to the nature of the studies described and providing context; and (4) change the section regarding human studies to give the reader better understanding that the studies were not designed to provide evidence of carcinogenicity. Further, a very brief synopsis of the drinking water studies showing an association between drinking water and cancer could also be added to the text, with the necessary caveats indicating the limitations of these studies with regard to establishing a causal relationship between DCA and human cancer.

Response: The recommended changes have been made with the following two exceptions: the cancer studies involving drinking water exposure have not been added and introductory paragraphs to every section in the hazard identification section have not been added due to space limitations. However, the format of each section has been revised to allow for greater integration of the data and to provide opportunities, in some cases, for cross-study comparisons.

APPENDIX B

SUMMARY OF STUDIES ON DCA TOXICITY AND APPLICABILITY FOR BMD ANALYSIS

APPENDIX B

Summary of Studies on DCA Toxicity and Applicability for BMD Analysis

DATA SUMMARY								SELECTION CRITERIA						
								Quantitative			Semi-Quant.		Qual.	
Endpoint	Species	Reference	Duration	Grps	NOAEL	LOAEL	Effect	>2 groups?	L <10*LL?	N <LL?	L not near max?	DR trend?	Tox. Rel?	BMD Model?
Metabol.	Human	Stacpoole et al., 1978	6-7 days	1		43	Decreased serum glucose, lactate	no	yes	--				no
		Moore et al., 1979	5-16 wks	1		50	Decreased cholesterol	no	yes	--				no
	Rat	Evans and Stacpoole, 1982	7 days	2		100	Decreased serum lactate	no	yes	--				no
		Davis, 1990	1 day	3	120		--	yes	yes	no				no
		Davis, 1986	2 weeks	5	150		--	yes	yes	no				no
		Katz et al., 1981	3 months	4		125	Decreased serum lactate and glucose	yes	no	--				no
		Yount et al., 1982	3 months	2		323	Altered serum metabolites	no	no	--				no
	Dog	Ribes et al., 1979	7 days	2		150	Decreased serum metabolites	no	no	--				no
		Katz et al., 1981	13 weeks	4		50	Decreased serum metabolites	yes	yes	--			no	no
Hepatic	Human	Stacpoole et al., 1998	? months	1		25	Increased serum enzymes	no	yes	--				no
	Mouse	Sanchez and Bull, 1990	2 weeks	4	57	190	Increased glycogen, focal necrosis	yes	no	no				no
		Kato-Weinstein et al., 1998	2 months	7		100	Increased glycogen	yes	yes	--			no	no
		Bull et al., 1990	1 year	3		140	Increased glycogen	yes	no	--				no
		DeAngelo et al., 1991	60-75 wks	3	7.6	77	Increased relative liver weight	yes	yes	yes			no	no
		Daniel et al., 1992	2 years	2		88	Increased liver weight, necrosis	no	yes	--				no
	Rat	Mather et al., 1990	3 months	4	3.9	35.5	Increased liver weight, glycogen	yes	yes	yes			yes	yes
		DeAngelo et al., 1996	100 weeks	3	40.2		--	yes	yes	no				no
		Katz et al., 1981	3 months	4		125	Increased liver weight	yes	no	--				no
		Smith et al., 1992	gestation	4	14	140	Increased liver weight	yes	no	no				no
		Toth et al., 1992	10 weeks	4		31	Increased liver weight	yes	yes	--			no	no
		Yount et al., 1982	3 months	2		323	Hepatomegaly	no	no	--				no
	Dog	Cicmanec et al., 1991	3 months	4		12.5	Increased liver weight	yes	yes	--		no		no
		Cicmanec et al., 1991	3 months	4		12.5	Liver histopathology	yes	yes	--	no	no		no
Neuro.	Human	Moore et al., 1979	5-16 weeks	1		50	Tingling, slowed NCV, poor reflexes	no	yes	--				no
		Spruijt et al., 2001	1 year	1		50	Peripheral neuropathy, slowed NCV	no	yes	--				no
		Stacpoole et al., 1998	<5 years	1		25	Sedation, peripheral neuropathy	no	yes	--				no
		Stacpoole et al., 1978	6-7 days	1		43	Sedation, peripheral neuropathy	no	yes	--				no
	Rat	Yount et al., 1982	3 months	2		323	Slowed NCV, hindlimb weakness	no	no	--				no
		Katz et al., 1981	3 months	4		125	Histological brain lesions	yes	no	--				no
	Dog	Cicmanec et al., 1991	3 months	4		12.5	Vacuolar changes in brain	yes	yes	--		no		no
		Katz et al., 1981	13 weeks	4		50	Vacuolar changes in brain	yes	yes	--		no		no
Repro.	Rat	Smith et al., 1992	g.d. 6-15	4	14	140	Decreased fetal wt, increased resorptions	yes	no	no				no
		Linder et al., 1997	2 weeks	5		54	Multiple effects on sperm formation	yes	yes	--				yes
		Toth et al., 1992	10 weeks	4		31	Decreased epididymal weight	yes	yes	--				yes
		DeAngelo et al., 1996	100 weeks	3	3.6	40.2	Increased testicular weight	yes	yes	yes		no		no
		Yount et al., 1982	3 months	2		323	Testicular degeneration	no	no	--				no
		Katz et al., 1981	13 weeks	4	125	500	Testicular degeneration	yes	no	no		no		no
	Dog	Katz et al., 1981	13 weeks	4		50	Prostate atrophy, testicular changes	yes	yes	--		no		no
		Cicmanec et al., 1991	3 months	4		12.5	Incidence of testicular lesions	yes	yes	--				yes

APPENDIX C

BENCHMARK DOSE-RESPONSE FOR NONCANCER ENDPOINTS

Noncancer BMD Modeling Results

Cicmanec et al., 1991

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 ====================================================================
     Quantal Quadratic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:55 $
     Input Data File: H:\OW\DCA\FINALBMD\NONCANCE\CICMANEC\CICMANEC.(d)
     Gnuplot Plotting File:  H:\OW\DCA\FINALBMD\NONCANCE\CICMANEC\CICMANEC.plt
                                    Wed Feb 21 14:33:51 2001
 ====================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^2)]

   Dependent variable = Affected
   Independent variable = Dose

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     Background = 0.0833333
                          Slope =  0.000462557
                          Power =  2 Specified

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Power
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

                  Slope

     Slope            1


                          Parameter Estimates

       Variable           Estimate             Std. Err.
     Background                  0                   NA
          Slope          0.0103004           0.00572423

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


                        Analysis of Deviance Table

       Model      Log(likelihood)    Deviance    Test DF    P-value
     Full model         -2.50201
   Fitted model         -2.50201    1.04799e-006      3           1
  Reduced model         -12.2173       19.4305        3      0.0002227

           AIC: 7.00403

Goodness  of  Fit

                                                                Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000      0.0000          0.000          0            5            0
   12.5000      0.8000          4.000          4            5   -4.68e-006
   39.5000      1.0000          5.000          5            5    0.0007239
   72.0000      1.0000          5.000          5            5            0

Chi-square = 0.00     DF = 3        P-value = 1.0000

Benchmark Dose Computation
```

```
Specified effect =            0.1
Risk Type =            Extra risk
Confidence level =           0.95
             BMD =        3.19824
            BMDL =       2.09228
```

Linder et al., 1997 (A2 Run)

```
=====================================================================
     Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
     Input Data File: H:\OW\DCA\FINALBMD\NONCANCE\LINDER97\COUNT\LIND97A2.(d)
     Gnuplot Plotting File:  H:\OW\DCA\FINALBMD\NONCANCE\LINDER97\COUNT\LIND97A2.plt
                                       Wed Feb 21 15:59:00 2001
 =====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)

   Dependent variable = MEAN
   Independent variable = Dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = alpha * mean(i) ^ rho

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
                         alpha =     0.639066
                           rho =      1.35894
                     intercept =          224
                             v =         -138
                             n =      1.55811
                             k =      214.237

          Asymptotic Correlation Matrix of Parameter Estimates
```

	alpha	rho	intercept	v	n	k
alpha	1	-1	-0.032	0.12	0.24	-0.1
rho	-1	1	0.028	-0.12	-0.24	0.1
intercept	-0.032	0.028	1	-0.72	-0.46	-0.39
v	0.12	-0.12	-0.72	1	0.79	-0.2
n	0.24	-0.24	-0.46	0.79	1	-0.2
k	-0.1	0.1	-0.39	-0.2	-0.2	1

```
                        Parameter Estimates

       Variable           Estimate            Std. Err.
          alpha            2.94166             8.45724
            rho            1.09046            0.564024
      intercept            236.288             9.20067
              v           -158.548               16.23
              n            1.47093            0.377618
              k            179.513             37.1316

     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean    Obs Std Dev   Est Mean   Est Std Dev   Chi^2 Res.
------     ---   --------    -----------   --------   -----------  ----------
   0     8        224           40          236         33.8         -0.364
```

18	8	248	32	231	33.4	0.507
54	8	208	25	213	31.9	-0.161
160	8	165	21	164	27.6	0.0468
480	8	106	35	108	22	-0.0883
1440	8	86	11	84.8	19.3	0.0609

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-182.377837	7	378.755674
A2	-175.765302	12	375.530604
A3	-181.171992	8	378.343984
fitted	-182.888696	6	377.777393
R	-225.464414	2	454.928827

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	99.3982	10	<.0001
Test 2	13.2251	5	0.02136
Test 3	10.8134	4	0.02874
Test 4	3.43341	2	0.1797

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .05. A non-homogeneous variance
model appears to be appropriate.

The p-value for Test 3 is less than .05. You may want to consider a
different variance model.

The p-value for Test 4 is greater than .05. The model chosen seems
to adequately describe the data.

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 73.8032

Warning: optimum may not have been found. Bad completion code in Optimization routine.

BMDL computation failed.

Linder et al., 1997 (B Run)

```
 ========================================================================
     Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
     Input Data File: H:\OW\DCA\FINALBMD\NONCANCE\LINDER97\MOTILITY\LINDE97B.(d)
     Gnuplot Plotting File:  H:\OW\DCA\FINALBMD\NONCANCE\LINDER97\MOTILITY\LINDE97B.plt
                                    Wed Feb 21 16:05:43 2001
 ========================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)

   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                          alpha =      110.154
                            rho =            0 Specified
                      intercept =           72
                              v =          -66
                              n =      1.05789
                              k =      190.476

           Asymptotic Correlation Matrix of Parameter Estimates

                 alpha         rho    intercept           v           n           k
    alpha            1           0           0           0           0           0
      rho            0           1           0           0           0           0
intercept            0           0           1           0           0           0
        v            0           0           0           1           0           0
        n            0           0           0           0           1           0
        k            0           0           0           0           0           1

                           Parameter Estimates

       Variable           Estimate             Std. Err.
          alpha              124.88                    1
            rho                   0                    1
      intercept              74.658                    1
              v             -67.6049                    1
              n              1.86631                    1
              k              177.165                    1

     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean    Obs Std Dev   Est Mean   Est Std Dev   Chi^2 Res.
------     ---   --------    -----------   --------   -----------   ----------
    0     8         72           12          74.7         11.2         -0.238
   18     8         74            8          73.7         11.2         0.0247
   54     8         72            9            68         11.2          0.356
  160     8         41           13          44.1         11.2         -0.274
  480     8         20           17          16.2         11.2          0.344
 1440     8          6            7          8.38         11.2         -0.213

 Model Descriptions for likelihoods calculated

 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model  R:         Yi = Mu + e(i)
```

```
           Var{e(i)} = Sigma^2

                     Likelihoods of Interest

           Model      Log(likelihood)   DF        AIC
            A1         -138.103400       7     290.206800
            A2         -133.640412      12     291.280823
          fitted       -139.856491       5     289.712983
             R         -186.484888       2     376.969776

Test 1:  Does response and/or variances differ among dose levels
         (A2 vs. R)
Test 2:  Are Variances Homogeneous (A1 vs A2)
Test 3:  Does the Model for the Mean Fit (A1 vs. fitted)

                      Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df     p-value
   Test 1               96.763         10          <.0001
   Test 2              8.92598          5          0.1121
   Test 3              3.50618          2          0.1732

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels. It seems appropriate to
model the data.

The p-value for Test 2 is greater than .05.  A homogeneous variance model appears to be
appropriate here.

The p-value for Test 3 is greater than .05.  The model chosen appears
to adequately describe the data

  Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95
             BMD =       74.3974
            BMDL =       46.8201
```

Mather et al., 1990

```
 ======================================================================
     Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
     Input Data File: H:\OW\DCA\FINALBMD\NONCANCE\MATHER\MATHER4.(d)
     Gnuplot Plotting File:  H:\OW\DCA\FINALBMD\NONCANCE\MATHER\MATHER4.plt
                                    Wed Feb 21 16:28:03 2001
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)

   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                          alpha =   0.00948683
                            rho =            0   Specified
                      intercept =          3.8
```

```
                              v =         2.62
                              n =     0.318455
                              k =      584.908

        Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -n
                 have been estimated at a boundary point, or have been specified by the
user,
                 and do not appear in the correlation matrix )

                    alpha          rho    intercept            v            k
     alpha              1            0            0            0            0
       rho              0            1            0            0            0
 intercept              0            0            1            0            0
         v              0            0            0            1            0
         k              0            0            0            0            1

                            Parameter Estimates

       Variable           Estimate             Std. Err.
          alpha           0.0116056                    1
            rho                   0                    1
      intercept             3.86856                    1
              v             3.26005                    1
              n                   1                   NA
              k             96.1449                    1

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.

     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean    Obs Std Dev   Est Mean   Est Std Dev   Chi^2 Res.
------     ---   --------    -----------   --------   -----------   ----------
    0    10        3.8          0.1         3.87        0.108         -0.636
  3.9    10       4.08          0.1            4        0.108          0.783
 35.5    10       4.73         0.09         4.75        0.108         -0.164
  345    10       6.42          0.1         6.42        0.108         0.0176

 Model Descriptions for likelihoods calculated

 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2
 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2

Degrees of freedom for Test A1 vs fitted <= 0

                       Likelihoods of Interest

            Model      Log(likelihood)   DF        AIC
              A1           75.183917       5    -140.367835
              A2           75.264219       8    -134.528438
           fitted          69.125316       5    -128.250632
              R           -21.361630       2      46.723261

 Test 1:  Does response and/or variances differ among dose levels
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous (A1 vs A2)
 Test 3:  Does the Model for the Mean Fit (A1 vs. fitted)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df     p-value
   Test 1              193.091          6          <.0001
   Test 2             0.160603          3          0.9837
   Test 3              12.1172          0              NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data
```

```
The p-value for Test 2 is greater than .05.  A homogeneous variance
model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0.  The Chi-Square
     test for fit is not valid

 Benchmark Dose Computation
Specified effect =            1

Risk Type        =     Estimated standard deviations from the control mean
Confidence level =          0.95
             BMD =       3.28571
            BMDL =       3.00326
```

Toth et al., 1992 (A Run)

```
 ======================================================================
    Linear Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
     Input Data File: H:\OW\DCA\FINALBMD\NONCANCE\TOTH92\COUNT\TOTH92A.(d)
     Gnuplot Plotting File:  H:\OW\DCA\FINALBMD\NONCANCE\TOTH92\COUNT\TOTH92A.plt
                                       Wed Feb 21 15:02:38 2001
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

   Dependent variable = MEAN
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = alpha*mean(i)^rho

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
                         alpha =            1
                           rho =            0
                        beta_0 =        638.2
                        beta_1 =     -2.14309

                         Parameter Estimates

       Variable           Estimate             Std. Err.
          alpha          0.00134237           0.0067082
            rho             2.64948            0.801147
         beta_0             639.904             30.5081
         beta_1            -2.17205            0.320949

           Asymptotic Correlation Matrix of Parameter Estimates

                  alpha          rho       beta_0       beta_1
     alpha            1           -1       -0.015        0.017
       rho           -1            1        0.015       -0.017
    beta_0       -0.015        0.015            1        -0.87
    beta_1        0.017       -0.017        -0.87            1

     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean    Obs Std Dev   Est Mean   Est Std Dev   Chi^2
Res.
------     ---   --------    -----------   --------   -----------   ----------
    0    19        630          205          640          191         -0.985
31.25    18        583          137          572          165            1.2
 62.5    18        503          164          504          139         -0.149
  125    19        368         91.6          368           92        -0.0821
```

```
 Model Descriptions for likelihoods calculated

 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2
 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = alpha*(Mu(i))^rho
 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                     Likelihoods of Interest

            Model      Log(likelihood)   DF        AIC
              A1         -408.202121      5     826.404241
              A2         -402.269507      8     820.539015
              A3         -403.341780      6     818.683559
           fitted        -403.457293      4     814.914586
              R          -422.268100      2     848.536200

                   Explanation of Tests

 Test 1:  Does response and/or variances differ among Dose
 levels?  (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value
   Test 1              39.9972          6          <.0001
   Test 2              11.8652          3        0.007859
   Test 3              2.14454          2          0.3422
   Test 4             0.231027          2          0.8909

The p-value for Test 1 is less than .05.  There appears to be a difference between response
and/or variances among the dose levels It seems appropriate to model the data.

The p-value for Test 2 is less than .05. A non-homogeneous variance
model appears to be appropriate.

The p-value for Test 3 is greater than .05. The modeled variance appears to be appropriate
here.

The p-value for Test 4 is greater than .05.  The model chosen seems to adequately describe
the data.

 Benchmark Dose Computation
Specified effect =           450

Risk Type        =     Point risk
Confidence level =          0.95
             BMD =       87.4306
            BMDL =       75.7201
```

Toth et al., 1992 (C Run)

```
 ======================================================================
     Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
     Input Data File: H:\OW\DCA\FINALBMD\NONCANCE\TOTH92\MOTILITY\TOTH92C.(d)
     Gnuplot Plotting File:  H:\OW\DCA\FINALBMD\NONCANCE\TOTH92\MOTILITY\TOTH92C.plt
                                       Wed Feb 21 15:30:34 2001
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)
```

Dependent variable = MEAN
Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	115.908	
rho =	0	Specified
intercept =	54.6	
v =	-27.5	
n =	5.16921	
k =	59.6104	

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	intercept	v	n	k
alpha	1	0	0	0	0	0
rho	0	1	0	0	0	0
intercept	0	0	1	0	0	0
v	0	0	0	1	0	0
n	0	0	0	0	1	0
k	0	0	0	0	0	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	109.538	1
rho	0	1
intercept	54.6	1
v	-27.8588	1
n	6.017	1
k	60.7743	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	15	54.6	10.2	54.6	10.5	3.54e-007
31.25	14	54.1	11.2	54.1	10.5	3.56e-009
62.5	17	39.5	12	39.5	10.5	7.21e-008
125	19	27.1	9.8	27.1	10.5	-2.48e-007

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Degrees of freedom for Test A1 vs fitted <= 0

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-185.128961	5	380.257923
A2	-184.702137	8	385.404274
fitted	-185.128962	5	380.257923
R	-211.659863	2	427.319726

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value

Test 1	53.0618	6	<.0001
Test 2	0.853649	3	0.8366
Test 3	5.57208e-007	0	NA

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance
model appears to be appropriate here.

NA - Degrees of freedom for Test 3 are less than or equal to 0. The Chi-Square
test for fit is not valid.

Benchmark Dose Computation

Specified effect	=	1
Risk Type	=	Estimated standard deviations from the control mean
Confidence level	=	0.95
BMD	=	55.8547
BMDL	=	40.3906

APPENDIX D

BENCHMARK DOSE-RESPONSE FOR CANCER ENDPOINTS
DeAngelo et al., 1999 (5 Doses)

Cancer BMD Modeling Results–5 Doses

```
=====================================================================
          $Revision: 2.2 $ $Date: 2001/03/14 01:17:00 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSEGAMMA.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSEGAMMA.plt
                                                  Sat Aug 03 09:50:30 2002
 =====================================================================

 DCA DeAngelo (1999) Five Doses
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cumulative Gamma distribution function

   Dependent variable = Either
   Independent variable = HED
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                 Default Initial (and Specified) Parameter Values
                    Background =     0.362745
                         Slope =    0.0528134
                         Power =          1.3

           Asymptotic Correlation Matrix of Parameter Estimates

             Background        Slope        Power
Background            1         0.19         0.26
     Slope         0.19            1         0.98
     Power         0.26         0.98            1

                         Parameter Estimates

       Variable           Estimate             Std. Err.
     Background            0.351081           0.0526875
          Slope            0.211892            0.137073
          Power              4.06938             2.80924

                       Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -85.1782
   Fitted model        -85.4032      0.449935      2          0.7985
  Reduced model        -111.914        53.471      4         <.0001
```

```
          AIC:         176.806

                    Goodness  of  Fit
                                                                  Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.3511         17.554         18           50       0.1321
    1.3000      0.3512         11.589         11           33      -0.2148
   13.2000      0.5420         13.550         14           25       0.1808
   26.5000      0.8713         30.497         30           35      -0.2509
   47.5000      0.9931         20.854         21           21       0.3829
 Chi-square =       0.31     DF = 2        P-value = 0.8582

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        8.45355
            BMDL =       2.54711



 ======================================================================
         Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSELOGISTIC.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSELOGISTIC.plt
                                                  Sat Aug 03 09:55:12 2002
 ======================================================================

 DCA DeAngelo (1999) Five Doses
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = 1/[1+EXP(-intercept-slope*dose)]

   Dependent variable = Either
   Independent variable = HED
   Slope parameter is not restricted

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                     background =            0   Specified
                      intercept =    -0.766205
                          slope =    0.0938121

          Asymptotic Correlation Matrix of Parameter Estimates
```

```
          ( *** The model parameter(s)  -background
                have been estimated at a boundary point, or have been specified by
                the user, and do not appear in the correlation matrix )

             intercept        slope
 intercept            1        -0.58
     slope        -0.58            1

                         Parameter Estimates

       Variable           Estimate             Std. Err.
      intercept           -0.734827            0.227439
          slope             0.09509           0.0171984

                       Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model         -85.1782
   Fitted model          -86.057       1.75755      3          0.6242
  Reduced model         -111.914        53.471      4         <.0001
           AIC:          176.114

                    Goodness  of  Fit

                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.3241         16.207         18           50       0.5418
    1.3000      0.3518         11.609         11           33      -0.2219
   13.2000      0.6272         15.681         14           25      -0.6952
   26.5000      0.8563         29.971         30           35      0.01387
   47.5000      0.9777         20.532         21           21       0.6916
 Chi-square =       1.30     DF = 3        P-value = 0.7280

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =         3.0997
            BMDL =        2.4293


=====================================================================
         Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSELOGLOGISTIC.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSELOGLOGISTIC.plt
                                              Sat Aug 03 09:57:01 2002
 =====================================================================

 DCA DeAngelo (1999) Five Doses
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:
```

```
  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

  Dependent variable = Either
  Independent variable = HED
  Slope parameter is not restricted

  Total number of observations = 5
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008

  User has chosen the log transformed model

                Default Initial Parameter Values
                   background =         0.36
                    intercept =     -5.15172
                        slope =      2.06624

          Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope
background            1        -0.27         0.23
 intercept        -0.27            1        -0.99
     slope         0.23        -0.99            1

                        Parameter Estimates

       Variable           Estimate            Std. Err.
     background            0.352308           0.0530284
      intercept            -9.86897             3.76293
          slope             3.46213             1.18873

                      Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -85.1782
   Fitted model         -85.726        1.0956      2          0.5782
  Reduced model        -111.914        53.471      4         <.0001

           AIC:         177.452

                     Goodness  of  Fit

                                                                 Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.3523         17.615         18           50       0.1139
    1.3000      0.3524         11.629         11           33      -0.2292
   13.2000      0.5348         13.369         14           25       0.2528
   26.5000      0.8796         30.786         30           35      -0.4082
   47.5000      0.9810         20.600         21           21       0.6383

 Chi-square = 0.70     DF = 2        P-value = 0.7034
```

```
   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk



=================================================================
         Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSELOGPROBIT.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSELOGPROBIT.plt
                                                Sat Aug 03 09:58:44 2002
 =================================================================

 DCA DeAngelo (1999) Five Doses
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function

   Dependent variable = Either
   Independent variable = HED
   Slope parameter is not restricted

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   User has chosen the log transformed model

                  Default Initial (and Specified) Parameter Values
                     background =         0.36
                      intercept =     -2.62547
                          slope =      1.05552

          Asymptotic Correlation Matrix of Parameter Estimates

            background    intercept        slope
background            1        -0.27         0.23
 intercept        -0.27            1        -0.99
     slope         0.23        -0.99            1

                         Parameter Estimates

       Variable           Estimate             Std. Err.
     background            0.351713           0.0527934
      intercept            -5.92321             2.15789
          slope             2.07572            0.677696
```

```
                    Analysis of Deviance Table
      Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -85.1782
   Fitted model        -85.5499      0.743422      2          0.6896
  Reduced model        -111.914        53.471      4         <.0001
           AIC:           177.1

                     Goodness  of  Fit
                                                                  Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.3517         17.586         18           50       0.1227
    1.3000      0.3517         11.607         11           33      -0.2211
   13.2000      0.5366         13.415         14           25       0.2344
   26.5000      0.8771         30.697         30           35      -0.3588
   47.5000      0.9881         20.751         21           21       0.5019
 Chi-square = 0.50     DF = 2        P-value = 0.7790

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        9.35739
            BMDL =       4.27367

=====================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSEMULTISTAGE2.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSEMULTISTAGE2.plt
                                                  Sat Aug 03 10:00:45 2002
 =====================================================================

 DCA DeAngelo (1999) Five Doses
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = Either
   Independent variable = HED

 Total number of observations = 5
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
```

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0
Beta(1) = 0
Beta(2) = 4.46493e+016

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1)
have been estimated at a boundary point, or have been specified by
the user, and do not appear in the correlation matrix)

	Background	Beta(2)
Background	1	-0.37
Beta(2)	-0.37	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.347888	0.0838624
Beta(1)	0	NA
Beta(2)	0.00223736	0.000611002

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-85.1782			
Fitted model	-85.3116	0.266877	3	0.9661
Reduced model	-111.914	53.471	4	<.0001

AIC: 174.623

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.3479	17.394	18	50	0.053
i: 2					
1.3000	0.3503	11.562	11	33	-0.075
i: 3					
13.2000	0.5584	13.960	14	25	0.006
i: 4					

```
   26.5000     0.8645        30.257        30          35      -0.063
i: 5
   47.5000     0.9958        20.912        21          21       1.004

 Chi-square = 0.18     DF = 3        P-value = 0.9809

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        6.86232
            BMDL =        2.05186



=====================================================================
         Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSEPROBIT.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSEPROBIT.plt
                                                Sat Aug 03 10:03:12 2002
 =====================================================================

 DCA DeAngelo (1999) Five Doses
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function

   Dependent variable = Either
   Independent variable = HED
   Slope parameter is not restricted

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     background =            0   Specified
                      intercept =    -0.442019
                          slope =    0.0558392

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -background
                 have been estimated at a boundary point, or have been specified by
                 the user, and do not appear in the correlation matrix )

              intercept        slope
```

```
 intercept            1         -0.6
     slope         -0.6            1

                         Parameter Estimates

       Variable           Estimate             Std. Err.
      intercept           -0.449908            0.138144
          slope           0.0570853          0.00973486

                      Analysis of Deviance Table
       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -85.1782
   Fitted model        -85.7683       1.18017      3          0.7578
  Reduced model        -111.914        53.471      4         <.0001
           AIC:         175.537

                    Goodness  of  Fit
                                                              Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.3264         16.319         18           50       0.5069
    1.3000      0.3536         11.668         11           33      -0.2432
   13.2000      0.6193         15.482         14           25      -0.6105
   26.5000      0.8561         29.963         30           35      0.01799
   47.5000      0.9881         20.751         21           21        0.502
 Chi-square = 0.94     DF = 3        P-value = 0.8155

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        3.15928
            BMDL =       2.53542



=======================================================================
         Quantal Linear Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSEQLINEAR.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSEQLINEAR.plt
                                                Sat Aug 03 10:04:30 2002
 =======================================================================

 DCA DeAngelo (1999) Five Doses
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose)]

   Dependent variable = Either
   Independent variable = HED
```

```
   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     Background =     0.362745
                          Slope =    0.0701811
                          Power =            1   Specified

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Power
                 have been estimated at a boundary point, or have been specified by
                 the user, and do not appear in the correlation matrix )

             Background        Slope
Background            1        -0.33
     Slope        -0.33            1

                          Parameter Estimates

       Variable           Estimate            Std. Err.
     Background             0.32128           0.0525609
          Slope            0.056121           0.0112913

                       Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model         -85.1782
   Fitted model         -87.2055       4.05467      3          0.2556
  Reduced model         -111.914        53.471      4         <.0001
           AIC:          178.411

                     Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.3213         16.064         18           50       0.5863
    1.3000      0.3690         12.178         11           33       -0.425
   13.2000      0.6764         16.911         14           25       -1.244
   26.5000      0.8466         29.631         30           35        0.173
   47.5000      0.9528         20.009         21           21         1.02

 Chi-square = 3.14     DF = 3        P-value = 0.3701

   Benchmark Dose Computation

Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        1.87738
            BMDL =        1.37335
```

```
================================================================
         Quantal Quadratic Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSEQQUADRATIC.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL
MODELING\5DOSEQQUADRATIC.plt
                                                Sat Aug 03 10:05:52 2002
 ================================================================

 DCA DeAngelo (1999) Five Doses
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^2)]

   Dependent variable = Either
   Independent variable = HED

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     Background =     0.362745
                          Slope =    0.0014775
                          Power =            2   Specified

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Power
                 have been estimated at a boundary point, or have been specified by
                 the user, and do not appear in the correlation matrix )

             Background        Slope
Background            1        -0.29
     Slope        -0.29            1

                          Parameter Estimates

       Variable           Estimate            Std. Err.
     Background             0.34789           0.0507344
          Slope          0.00223738         0.000540864

                        Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -85.1782
   Fitted model        -85.3116      0.266877      3          0.9661
  Reduced model        -111.914        53.471      4         <.0001

           AIC:         174.623
```

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3479	17.394	18	50	0.1798
1.3000	0.3504	11.562	11	33	-0.2049
13.2000	0.5584	13.960	14	25	0.01597
26.5000	0.8645	30.257	30	35	-0.1271
47.5000	0.9958	20.912	21	21	0.2972

Chi-square = 0.18 DF = 3 P-value = 0.9809

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 6.86229
BMDL = 5.69087

==========
Weibull Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL MODELING\5DOSEWEIBULL.(d)
Gnuplot Plotting File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\FINAL MODELING\5DOSEWEIBULL.plt
Sat Aug 03 10:06:59 2002
==========

DCA DeAngelo (1999) Five Doses

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]

Dependent variable = Either
Independent variable = HED
Power parameter is not restricted

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
Background = 0.362745
Slope = 0.00510196
Power = 1.67901

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope	Power

Background	1	-0.31	0.29
Slope	-0.31	1	-1
Power	0.29	-1	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.35075	0.0529332
Slope	0.00136633	0.00364981
Power	2.15173	0.817672

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-85.1782			
Fitted model	-85.2928	0.229231	2	0.8917
Reduced model	-111.914	53.471	4	<.0001
AIC:	176.586			

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3507	17.537	18	50	0.1371
1.3000	0.3523	11.626	11	33	-0.2282
13.2000	0.5435	13.587	14	25	0.166
26.5000	0.8659	30.308	30	35	-0.1529
47.5000	0.9974	20.946	21	21	0.2319

Chi-square = 0.18 DF = 2 P-value = 0.9160

Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	7.53401
BMDL =	2.50369

APPENDIX E

BENCHMARK DOSE-RESPONSE FOR CANCER ENDPOINTS
DeAngelo et al., 1999 (4 and 6 Doses)

Cancer BMD Modeling Results – 4 and 6 Dose Groups

As discussed in Section 5.3, the BMDL was estimated using data on the numbers of animals with either hepatocarcinoma or hepatoadenoma from the DeAngelo et al. (1999) study. The highest dose group was excluded from the main analysis because the highest dose in the study (64.6 mg/kg-day HED) resulted in reduced weight gain over the second half of the study and a greatly increased severity of hepatic necrosis in test animals. Based on these findings, it was judged that the highest dose was at or near the MTD.

For purposes of comparison, this appendix presents the results of BMD modeling when all dose groups are included. In addition, BMDL modeling has been performed with the two highest dose groups excluded. This approach was taken because the prevalence of combined hepatocarcinoma and adenoma in the second highest dose group was 100%, and omitting this group (and the highest-dose group) could result in a better model fit in the low-dose range.

Results of the BMD modeling using all six dose groups and the four lowest dose groups are summarized in Tables E-1 and E-2. When all six dose groups are used, the results are very similar to those presented in Section 5.3 for modeling with the highest dose omitted. The multistage and quantal-quadratic models estimate identical BMDs and have identical p-values and AICs. The BMDL estimated using the multistage model with all six dose groups is again 2.1 mg/kg-day (rounded up from 2.08), essentially the same as that obtained when the high dose group was omitted (2.1 mg/kg-day, rounded from 2.05). The BMDLs estimated from the good-fitting models (p-values greater than 0.8) range from 2.08 (multistage) to 5.69 mg/kg-day (quantal-quadratic).

Table E-1. Results of benchmark dose carcinogenicity including all (six) dose groups [1,2]

Model	BMD	BMDL	p-value	AIC
Multi-Stage(2)	6.86	2.08	0.996	174.63
Quantal-quadr.	6.86	5.69	0.996	174.63
Weibull	7.54	2.61	0.981	176.59
Gamma	8.49	2.73	0.957	176.82
Probit	3.15	2.53	0.917	175.55
Log-Probit	9.46	4.62	0.910	177.14
Logistic	3.08	2.42	0.849	176.21
Log-Logistic	9.37	4.51	0.846	177.59

Quantal-linear	1.83	1.35	0.489	178.79

1. Data = DeAngelo, et al. (1999), animals with hepatocarcinoma or adenoma
2. BMD estimated using BMR = 0.10, BMDL estimated as 95% UCL

When the two highest dose groups are omitted, the multistage and quantal-quadratic model again provide the best fits to the data (p-value = 0.962, AIC = 174.43). On the whole, the p-values are slightly lower than when five or six dose groups are used. In this case, the multistage (as well as the gamma, Weibull, and log-logistic models) predict a BMDL of 1.7 mg/kg-day. The other four good-fitting models (p-value above 0.7) predict BMDLs of 1.83 (log-probit), 2.59 (probit), 2.51 (logistic), and 5.72 mg/kg-day (quantal-quadratic).

Table E-2. Results of benchmark dose carcinogenicity excluding the two highest dose groups [1,2]

Model	BMD	BMDL	p-value	AIC
Multi-Stage(2)	6.96	1.70	0.962	174.43
Quantal-quadr.	6.96	5.72	0.962	174.43
Log-Probit	8.28	1.83	0.802	176.42
Log-Logistic	7.95	1.73	0.800	176.42
Gamma	7.49	1.70	0.800	176.42
Weibull	6.78	1.70	0.783	176.43
Probit	3.30	2.59	0.743	174.95
Logistic	3.28	2.51	0.714	175.03
Quantal-linear	2.17	1.50	0.439	176.01

1. Data = DeAngelo, et al. (1999), animals with hepatocarcinoma or adenoma, omitting two highest dose groups (control and three dosed groups)
2. BMD estimated using BMR = 0.10, BMDL estimated as 95% UCL

Including the highest dose group therefore has very little effect on the estimated BMDL. Excluding the two highest dose groups, results in the bulk of the models estimate BMDLs that are 15 to 20% lower than the multistage model estimate when the single highest dose group is excluded. As noted in Section 5.3, because of the lack of conclusive evidence concerning the carcinogenic mode of action of DCA, the quantal-quadratic model, which assumes zero slope at zero dose, was not considered to provide reliable BMDL estimates, despite its good fit to the data.

Benchmark Modeling Results for Entire Data Set (6 Dose Groups)

```
=====================================================================
          $Revision: 2.2 $ $Date: 2001/03/14 01:17:00 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA_ALL_TUMORS.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA_ALL_TUMORS.plt
                                                  Thu Aug 08 10:57:03 2002
 =====================================================================

 UBMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cumulative Gamma distribution function

   Dependent variable = Either
   Independent variable = HED
   Power parameter is restricted as power >=1

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                 Default Initial (and Specified) Parameter Values
                    Background =     0.362745
                         Slope =    0.0528134
                         Power =          1.3

          Asymptotic Correlation Matrix of Parameter Estimates

            Background        Slope        Power
Background            1         0.19         0.26
     Slope         0.19            1         0.98
     Power         0.26         0.98            1

                         Parameter Estimates

       Variable           Estimate             Std. Err.
     Background            0.351181           0.0526892
          Slope            0.213843            0.135637
          Power             4.10684             2.78823

                       Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -85.1782
   Fitted model        -85.4078      0.459312      3          0.9277
  Reduced model        -117.777       65.1977      5         <.0001
```

AIC: 176.816

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3512	17.559	18	50	0.1306
1.3000	0.3513	11.592	11	33	-0.2159
13.2000	0.5411	13.529	14	25	0.1891
26.5000	0.8718	30.513	30	35	-0.2593
47.5000	0.9932	20.858	21	21	0.3782
64.6000	0.9996	10.995	11	11	0.06768

Chi-square = 0.31 DF = 3 P-value = 0.9573

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 8.49415
BMDL = 2.73397

==========
Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\DCA_ALL_TUMORS.(d)
Gnuplot Plotting File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\DCA_ALL_TUMORS.plt
Thu Aug 08 10:59:14 2002
==========

UBMDS MODEL RUN
~~~~~~~~~~

The form of the probability function is:

P[response] = 1/[1+EXP(-intercept-slope*dose)]

Dependent variable = Either
Independent variable = HED
Slope parameter is not restricted

Total number of observations = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
~~~~~~~~~~

```
                  Default Initial Parameter Values
                     background =            0   Specified
                      intercept =    -0.493007
                          slope =    0.0690064

          Asymptotic Correlation Matrix of Parameter Estimates

        ( *** The model parameter(s)  -background
              have been estimated at a boundary point, or have been specified by the
              user, and do not appear in the correlation matrix )

             intercept        slope
 intercept            1        -0.57
     slope        -0.57            1

                         Parameter Estimates

       Variable           Estimate             Std. Err.
      intercept           -0.739275            0.226806
          slope           0.0958841           0.0169284

                       Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -85.1782
   Fitted model         -86.105       1.85361      4          0.7627
  Reduced model        -117.777       65.1977      5         <.0001

           AIC:          176.21

                     Goodness  of  Fit

                                                                  Scaled
    Dose     Est._Prob.    Expected    Observed     Size        Residual
  ---------------------------------------------------------------------
    0.0000      0.3232         16.158         18           50         0.557
    1.3000      0.3510         11.583         11           33       -0.2127
   13.2000      0.6286         15.716         14           25       -0.7103
   26.5000      0.8584         30.042         30           35      -0.02049
   47.5000      0.9784         20.547         21           21        0.6802
   64.6000      0.9957         10.953         11           11        0.2169

 Chi-square =       1.37     DF = 4        P-value = 0.8494


   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        3.08205
            BMDL =       2.42162



=====================================================================
```

```
        Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
        Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA_ALL_TUMORS.(d)
        Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA_ALL_TUMORS.plt
                                                  Thu Aug 08 10:58:15 2002
 ========================================================================

 UBMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   Dependent variable = Either
   Independent variable = HED
   Slope parameter is restricted as slope >= 1

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   User has chosen the log transformed model

                 Default Initial Parameter Values
                    background =         0.36
                     intercept =     -5.08987
                         slope =      2.02139

          Asymptotic Correlation Matrix of Parameter Estimates

            background    intercept        slope
background           1        -0.28         0.24
 intercept       -0.28            1        -0.99
     slope        0.24        -0.99            1

                         Parameter Estimates

       Variable           Estimate             Std. Err.
     background             0.35305           0.0531767
      intercept            -10.1683             3.73822
          slope             3.56171              1.1728

                      Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model         -85.1782
   Fitted model         -85.7956       1.23487      3          0.7447
  Reduced model         -117.777       65.1977      5         <.0001

          AIC:         177.591
```

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3531	17.653	18	50	0.1028
1.3000	0.3531	11.653	11	33	-0.2377
13.2000	0.5298	13.245	14	25	0.3024
26.5000	0.8824	30.883	30	35	-0.463
47.5000	0.9825	20.632	21	21	0.6116
64.6000	0.9940	10.934	11	11	0.2568

Chi-square = 0.81 DF = 3 P-value = 0.8464

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 9.37448
BMDL = 4.51405

==========
Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\DCA_ALL_TUMORS.(d)
Gnuplot Plotting File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\DCA_ALL_TUMORS.plt
Thu Aug 08 11:01:31 2002
==========

UBMDS MODEL RUN
~~~~~~~~~~

The form of the probability function is:

P[response] = Background
+ (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

where CumNorm(.) is the cumulative normal distribution function

Dependent variable = Either
Independent variable = HED
Slope parameter is not restricted

Total number of observations = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
~~~~~~~~~~

```
   User has chosen the log transformed model

                Default Initial (and Specified) Parameter Values
                   background =         0.36
                    intercept =     -2.62162
                        slope =       1.0525

          Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope
background            1        -0.27         0.24
 intercept        -0.27            1        -0.99
     slope         0.24        -0.99            1

                         Parameter Estimates

       Variable           Estimate            Std. Err.
     background           0.352048           0.0528377
      intercept            -6.0128             2.11645
          slope            2.10539            0.661561



                      Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -85.1782
   Fitted model        -85.5714      0.786451      3          0.8527
  Reduced model        -117.777       65.1977      5         <.0001

           AIC:         177.143

                    Goodness  of  Fit
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.3520         17.602         18           50       0.1177
    1.3000      0.3520         11.618         11           33      -0.2251
   13.2000      0.5340         13.350         14           25       0.2606
   26.5000      0.8785         30.746         30           35      -0.3859
   47.5000      0.9889         20.766         21           21       0.4864
   64.6000      0.9981         10.980         11           11        0.143
 Chi-square =       0.54     DF = 3        P-value = 0.9104

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        9.46131
            BMDL =        4.6164



=================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
```

```
        Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA_ALL_TUMORS.(d)
        Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA_ALL_TUMORS.plt
                                                  Thu Aug 08 11:02:47 2002
 ========================================================================

 UBMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = Either
   Independent variable = HED

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) = 5.62186e+017
                        Beta(2) = 1.98607e+016

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(1)
                 have been estimated at a boundary point, or have been specified by
                 the user, and do not appear in the correlation matrix )


             Background      Beta(2)
Background            1        -0.37
   Beta(2)        -0.37            1

                         Parameter Estimates

       Variable           Estimate            Std. Err.
     Background           0.347869           0.0838449
        Beta(1)                  0                  NA
        Beta(2)         0.00223812         0.000610001

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
```

```
     has no standard error.

                        Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model         -85.1782
   Fitted model         -85.3123       0.26814      4          0.9918
  Reduced model         -117.777       65.1977      5         <.0001

           AIC:          174.625

                      Goodness  of  Fit

     Dose     Est._Prob.    Expected    Observed     Size     Chi^2 Res.
  ----------------------------------------------------------------------
i: 1
    0.0000     0.3479        17.393        18          50       0.053
i: 2
    1.3000     0.3503        11.561        11          33      -0.075
i: 3
   13.2000     0.5585        13.961        14          25       0.006
i: 4
   26.5000     0.8646        30.260        30          35      -0.063
i: 5
   47.5000     0.9958        20.912        21          21       1.004
i: 6
   64.6000     0.9999        10.999        11          11       1.000

 Chi-square =       0.18     DF = 4        P-value = 0.9962

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        6.86115
            BMDL =        2.07845



=======================================================================
         Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA_ALL_TUMORS.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA_ALL_TUMORS.plt
                                                  Thu Aug 08 11:03:53 2002
 =======================================================================

 UBMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),
```

where CumNorm(.) is the cumulative normal distribution function

Dependent variable = Either
Independent variable = HED
Slope parameter is not restricted

Total number of observations = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
background = 0 Specified
intercept = -0.366862
slope = 0.0471876

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.6
slope	-0.6	1

Parameter Estimates

Variable	Estimate	Std. Err.
intercept	-0.45065	0.137888
slope	0.0572063	0.00964233

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-85.1782			
Fitted model	-85.7748	1.19325	4	0.8792
Reduced model	-117.777	65.1977	5	<.0001

AIC: 175.55

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3261	16.306	18	50	0.511
1.3000	0.3534	11.661	11	33	-0.2406
13.2000	0.6196	15.490	14	25	-0.614
26.5000	0.8566	29.982	30	35	0.008589
47.5000	0.9883	20.754	21	21	0.4987
64.6000	0.9994	10.994	11	11	0.08041

Chi-square = 0.95 DF = 4 P-value = 0.9171

```
   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        3.15463
            BMDL =       2.53418



=====================================================================
        Quantal Linear Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
        Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA_ALL_TUMORS.(d)
        Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA_ALL_TUMORS.plt
                                                  Thu Aug 08 11:05:32 2002
 =====================================================================

 UBMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose)]


   Dependent variable = Either
   Independent variable = HED

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     Background =     0.362745
                          Slope =    0.0422209
                          Power =            1   Specified

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Power
                 have been estimated at a boundary point, or have been specified by
                 the user, and do not appear in the correlation matrix )

             Background        Slope
Background            1        -0.32
     Slope        -0.32            1

                          Parameter Estimates

       Variable           Estimate            Std. Err.
     Background            0.319734           0.0523578
          Slope           0.0576209           0.0110511
```

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-85.1782			
Fitted model	-87.3967	4.43708	4	0.3501
Reduced model	-117.777	65.1977	5	<.0001

AIC: 178.793

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3197	15.987	18	50	0.6105
1.3000	0.3688	12.171	11	33	-0.4226
13.2000	0.6821	17.051	14	25	-1.31
26.5000	0.8522	29.829	30	35	0.08159
47.5000	0.9559	20.075	21	21	0.9838
64.6000	0.9836	10.819	11	11	0.4289

Chi-square = 3.43 DF = 4 P-value = 0.4890

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 1.82851
BMDL = 1.35289

==========
Quantal Quadratic Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\DCA_ALL_TUMORS.(d)
Gnuplot Plotting File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\DCA_ALL_TUMORS.plt
Thu Aug 08 11:06:41 2002
==========

UBMDS MODEL RUN
~~~~~~~~~~

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-slope*dose^2)]

Dependent variable = Either
Independent variable = HED

Total number of observations = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
~~~~~~~~~~

```
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                Default Initial (and Specified) Parameter Values
                   Background =     0.362745
                        Slope =  0.000653574
                        Power =            2   Specified

          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been specified by
                the user, and do not appear in the correlation matrix )

             Background        Slope
Background            1        -0.29
     Slope        -0.29            1

                        Parameter Estimates

       Variable           Estimate             Std. Err.
     Background            0.347869            0.050728
          Slope          0.00223812         0.000540196

                     Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model         -85.1782
   Fitted model         -85.3123       0.26814      4          0.9918
  Reduced model         -117.777       65.1977      5         <.0001

           AIC:         174.625

                    Goodness  of  Fit

                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000      0.3479         17.393         18           50       0.1801
    1.3000      0.3503         11.561         11           33      -0.2047
   13.2000      0.5585         13.961         14           25      0.01553
   26.5000      0.8646         30.260         30           35      -0.1282
   47.5000      0.9958         20.912         21           21       0.2969
   64.6000      0.9999         10.999         11           11       0.0251

 Chi-square =       0.18     DF = 4        P-value = 0.9962


   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        6.86116
            BMDL =       5.69064
```

```
================================================================
         Weibull Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA_ALL_TUMORS.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA_ALL_TUMORS.plt
                                                  Thu Aug 08 11:07:43 2002
 ================================================================

 UBMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]

   Dependent variable = Either
   Independent variable = HED
   Power parameter is restricted as power >=1

   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     Background =     0.362745
                          Slope =    0.0273825
                          Power =      1.10388

           Asymptotic Correlation Matrix of Parameter Estimates

             Background        Slope        Power
Background            1        -0.31         0.29
     Slope        -0.31            1           -1
     Power         0.29           -1            1

                          Parameter Estimates

       Variable          Estimate            Std. Err.
     Background           0.350763           0.0529253
          Slope         0.00136197          0.00362597
          Power            2.15274            0.814689

                        Analysis of Deviance Table
```

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-85.1782			
Fitted model	-85.293	0.229541	3	0.9727
Reduced model	-117.777	65.1977	5	<.0001

AIC: 176.586

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3508	17.538	18	50	0.1369
1.3000	0.3523	11.626	11	33	-0.2283
13.2000	0.5434	13.584	14	25	0.1668
26.5000	0.8660	30.309	30	35	-0.1534
47.5000	0.9975	20.947	21	21	0.2315
64.6000	1.0000	11.000	11	11	0.01242

Chi-square = 0.18 DF = 3 P-value = 0.9814

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 7.53803
BMDL = 2.61177

Benchmark Dose Modeling Results Excluding Two Highest Dose Groups
(Four Dose Groups)

==========

$Revision: 2.2 $ $Date: 2001/03/14 01:17:00 $
Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\DCA4HIGHEST.(d)
Gnuplot Plotting File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\DCA4HIGHEST.plt
Thu Jul 18 09:00:04 2002

==========

BMDS MODEL RUN

~~~~~~~~~~

The form of the probability function is:
P[response]= background+(1-background)*CumGamma[slope*dose,power],
~~~~~~~~~~

where CumGamma(.) is the cumulative Gamma distribution function

Dependent variable = Either
Independent variable = HED
Power parameter is restricted as power >=1

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
Background = 0.362745
Slope = 0.186832
Power = 3.70123

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope	Power
Background	1	0.17	0.22
Slope	0.17	1	0.99
Power	0.22	0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.349291	0.0525503
Slope	0.16719	0.140528
Power	3.24212	2.7498

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-85.1782			
Fitted model	-85.2105	0.0646347	1	0.7993
Reduced model	-99.0886	27.8208	3	<.0001

AIC: 176.421

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3493	17.465	18	50	0.1588
1.3000	0.3498	11.542	11	33	-0.198
13.2000	0.5595	13.987	14	25	0.005356
26.5000	0.8573	30.005	30	35	-0.002554

Chi-square = 0.06 DF = 1 P-value = 0.7996

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk

```
Confidence level =           0.95
             BMD =        7.49106
            BMDL =       1.69767



=====================================================================
         Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.plt
                                                Thu Aug 08 10:40:29 2002
 =====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = 1/[1+EXP(-intercept-slope*dose)]

   Dependent variable = Either
   Independent variable = HED
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                     background =            0   Specified
                      intercept =    -0.724563
                          slope =    0.0880096

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -background
                 have been estimated at a boundary point, or have been specified by
                 the user, and do not appear in the correlation matrix )

              intercept        slope
 intercept            1         -0.6
     slope        -0.6            1

                          Parameter Estimates

       Variable           Estimate            Std. Err.
      intercept           -0.701555            0.230098
          slope           0.0881974           0.0187968

                       Analysis of Deviance Table
```

```
       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -85.1782
   Fitted model        -85.5143      0.672212      2          0.7145
  Reduced model        -99.0886       27.8208      3         <.0001

           AIC:         175.029

                    Goodness  of  Fit

                                                                  Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000      0.3315         16.573         18           50       0.4286
    1.3000      0.3573         11.792         11           33      -0.2879
   13.2000      0.6136         15.341         14           25      -0.5508
   26.5000      0.8369         29.293         30           35       0.3235

 Chi-square =       0.67     DF = 2        P-value = 0.7137

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        3.27774
            BMDL =       2.51045



=====================================================================
         Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.plt
                                                Thu Jul 18 09:01:00 2002
 =====================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

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

   Dependent variable = Either
   Independent variable = HED
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   User has chosen the log transformed model
```

Default Initial Parameter Values
background = 0.36
intercept = -4.76605
slope = 1.72426

Asymptotic Correlation Matrix of Parameter Estimates

	background	intercept	slope
background	1	-0.21	0.17
intercept	-0.21	1	-0.99
slope	0.17	-0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0.349285	0.0524508
intercept	-8.17034	3.8887
slope	2.88043	1.26948

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-85.1782			
Fitted model	-85.2103	0.0642027	1	0.8
Reduced model	-99.0886	27.8208	3	<.0001

AIC: 176.421

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3493	17.464	18	50	0.1589
1.3000	0.3497	11.539	11	33	-0.1969
13.2000	0.5597	13.993	14	25	0.002802
26.5000	0.8572	30.003	30	35	-0.001644

Chi-square = 0.06 DF = 1 P-value = 0.8002

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 7.95414
BMDL = 1.73353

==========

Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\DCA4HIGHEST.(d)
Gnuplot Plotting File: C:\DOCUMENTS AND SETTINGS\06157\MY DOCUMENTS\DCA\DCA4HIGHEST.plt

Thu Aug 08 10:48:03 2002

BMDS MODEL RUN

The form of the probability function is:

P[response] = Background
+ (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

where CumNorm(.) is the cumulative normal distribution function

Dependent variable = Either
Independent variable = HED
Slope parameter is not restricted

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial (and Specified) Parameter Values
background = 0.36
intercept = -2.48935
slope = 0.92656

Asymptotic Correlation Matrix of Parameter Estimates

	background	intercept	slope
background	1	-0.21	0.17
intercept	-0.21	1	-0.99
slope	0.17	-0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0.349398	0.0523345
intercept	-5.01493	2.34977
slope	1.76634	0.763476

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-85.1782			
Fitted model	-85.2094	0.0623592	1	0.8028
Reduced model	-99.0886	27.8208	3	<.0001

AIC: 176.419

```
                    Goodness  of  Fit
                                                                  Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000      0.3494         17.470         18           50       0.1572
    1.3000      0.3494         11.530         11           33      -0.1936
   13.2000      0.5600         14.000         14           25   3.554e-005
   26.5000      0.8571         30.000         30           35  -2.898e-005

 Chi-square =       0.06     DF = 1        P-value = 0.8031


   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        8.27816
            BMDL =       1.83164



======================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.plt
                                                    Thu Jul 18 09:01:41 2002
 ======================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = Either
   Independent variable = HED

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008
```

```
                  Default Initial Parameter Values
                     Background =     0.345937
                        Beta(1) =   0.00210524
                        Beta(2) =   0.00208855

          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Beta(1)
                have been estimated at a boundary point, or have been specified by
                the user, and do not appear in the correlation matrix )

             Background      Beta(2)
Background            1        -0.39
   Beta(2)        -0.39            1

                         Parameter Estimates

       Variable           Estimate            Std. Err.
     Background           0.349311           0.0845452
        Beta(1)                  0              NA
        Beta(2)         0.00217333        0.000652057

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.

                       Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -85.1782
   Fitted model        -85.2172     0.0779541      2          0.9618
  Reduced model        -99.0886       27.8208      3         <.0001

          AIC:         174.434

                    Goodness  of  Fit

     Dose     Est._Prob.    Expected    Observed     Size     Chi^2 Res.
  ------------------------------------------------------------------------
i: 1
    0.0000     0.3493        17.466        18          50       0.047
i: 2
    1.3000     0.3517        11.606        11          33      -0.081
i: 3
   13.2000     0.5544        13.861        14          25       0.023
i: 4
   26.5000     0.8586        30.050        30          35      -0.012
 Chi-square =       0.08     DF = 2        P-value = 0.9619
   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        6.96267
            BMDL =        1.69536
```

```
================================================================
         Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.plt
                                              Thu Aug 08 10:52:47 2002
 ================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function

   Dependent variable = Either
   Independent variable = HED
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     background =            0   Specified
                      intercept =    -0.429874
                          slope =    0.0538043

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -background
                 have been estimated at a boundary point, or have been specified by
            the user, and do not appear in the correlation matrix )



              intercept        slope
 intercept            1        -0.61
     slope        -0.61            1

                           Parameter Estimates

       Variable           Estimate             Std. Err.
      intercept           -0.433568            0.140472
          slope           0.0539456           0.0109001

                        Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
```

```
     Full model        -85.1782
   Fitted model        -85.4739      0.591454      2           0.744
  Reduced model        -99.0886       27.8208      3         <.0001

           AIC:         174.948

                    Goodness  of  Fit
                                                                 Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.3323         16.615         18           50       0.4158
    1.3000      0.3581         11.819         11           33      -0.2972
   13.2000      0.6097         15.242         14           25      -0.5093
   26.5000      0.8404         29.413         30           35       0.2709

 Chi-square =       0.59     DF = 2        P-value = 0.7430


   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =         3.2962
            BMDL =        2.58591



======================================================================
         Quantal Linear Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.plt
                                                Thu Jul 18 09:03:07 2002
 ======================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose)]

   Dependent variable = Either
   Independent variable = HED

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     Background =     0.362745
                          Slope =    0.0538938
```

Power = 1 Specified

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Power
have been estimated at a boundary point, or have been specified by
the user, and do not appear in the correlation matrix)

	Background	Slope
Background	1	-0.37
Slope	-0.37	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.328661	0.0533696
Slope	0.0485733	0.011993

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-85.1782			
Fitted model	-86.0023	1.64815	2	0.4386
Reduced model	-99.0886	27.8208	3	<.0001

AIC: 176.005

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3287	16.433	18	50	0.4718
1.3000	0.3697	12.201	11	33	-0.4333
13.2000	0.6464	16.161	14	25	-0.9038
26.5000	0.8147	28.514	30	35	0.6465

Chi-square = 1.65 DF = 2 P-value = 0.4393

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 2.16911
BMDL = 1.49987

==========

Quantal Quadratic Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.(d)
Gnuplot Plotting File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.plt
Thu Jul 18 09:04:01 2002

```
 ================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^2)]

   Dependent variable = Either
   Independent variable = HED

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     Background =     0.362745
                          Slope =   0.00203373
                          Power =            2   Specified

           Asymptotic Correlation Matrix of Parameter Estimates

                  ( *** The model parameter(s) -Power
                        have been estimated at a boundary point, or have been
                        specified by the user, and do not appear in the correlation
                        matrix )

              Background        Slope
Background             1        -0.31
     Slope         -0.31            1

                          Parameter Estimates

       Variable           Estimate             Std. Err.
     Background            0.349311           0.0510221
          Slope          0.00217333         0.000565508

                        Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model         -85.1782
   Fitted model         -85.2172     0.0779541      2          0.9618
  Reduced model         -99.0886       27.8208      3         <.0001

           AIC:          174.434

                     Goodness  of  Fit

                                                                  Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
  ----------------------------------------------------------------------
    0.0000      0.3493         17.466         18           50       0.1585
```

```
    1.3000      0.3517         11.606         11           33      -0.2209
   13.2000      0.5544         13.861         14           25       0.05602
   26.5000      0.8586         30.050         30           35      -0.02422

 Chi-square =       0.08     DF = 2        P-value = 0.9619

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        6.96267
            BMDL =       5.72293



 ======================================================================
         Weibull Model $Revision: 2.2 $ $Date: 2000/03/17 22:27:16 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\06157\MY
DOCUMENTS\DCA\DCA4HIGHEST.plt
                                                  Thu Jul 18 09:04:51 2002
 ======================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]

   Dependent variable = Either
   Independent variable = HED
   Power parameter is restricted as power >=1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     Background =     0.362745
                          Slope =   0.00220968
                          Power =      1.97468



          Asymptotic Correlation Matrix of Parameter Estimates

             Background        Slope        Power
Background            1         -0.3         0.28
```

Slope	-0.3	1	-1
Power	0.28	-1	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.34865	0.0531378
Slope	0.00248422	0.00736373
Power	1.95791	0.930113

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-85.1782			
Fitted model	-85.2162	0.0759528	1	0.7829
Reduced model	-99.0886	27.8208	3	<.0001
AIC:	176.432			

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3487	17.433	18	50	0.1684
1.3000	0.3513	11.595	11	33	-0.2168
13.2000	0.5583	13.956	14	25	0.01761
26.5000	0.8575	30.013	30	35	-0.006207

Chi-square = 0.08 DF = 1 P-value = 0.7832

Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	6.78011
BMDL =	1.6957