

NATIONAL TOXICOLOGY PROGRAM

EXECUTIVE SUMMARY OF SAFETY AND TOXICITY INFORMATION

DICHLOROACETIC ACID

CAS Number 79-43-6

TRICHLOROACETIC ACID

CAS Number 76-03-9

September 30, 1991

Submitted to:

NATIONAL TOXICOLOGY PROGRAM

Submitted by:

Arthur D. Little, Inc.

Chemical Evaluation Committee Draft Report

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OVERVIEW 1

Nomination History: *Dichloroacetic Acid (DCA) and trichloroacetic Acid (TCA) were nominated by the U. S. Environmental Protection Agency (EPA) in 1988 for carcinogenicity testing, with a high priority. The nomination of these compounds was based on their potential for extensive human exposure as by-products of water disinfection and the suspicion that these compounds may be carcinogenic.*

Chemical and Physical Properties: *DCA is a colorless liquid with a pungent odor. It has a melting range of 9.0-13.5 °C (48.2-56.3 °F) and a boiling range of 193-194 °C (379.4-381.2 °F). DCA is soluble in water, alcohol, ether, and acetone. It is incompatible with strong oxidizing agents, bases, and reducing agents. Decomposition products from this compound include carbon monoxide, carbon dioxide, and hydrogen chloride.*

TCA is a solid which occurs as colorless, deliquescent crystals having a pungent odor. Its melting and boiling ranges are 57.0-58.0 °C (134.6-136.4 °F) and 196-197 °C (384.8-386.6 °F), respectively. TCA is soluble in water and most organic solvents including alcohol, acetone, and ether. It is incompatible with strong oxidizers and strong bases. Its decomposition results in the formation of chloroform, alkali carbonate, hydrochloric acid, carbon dioxide, carbon monoxide, and hydrogen chloride gas.

Production/Uses/Exposure: *DCA and TCA are disinfection by-products formed by the chlorination of natural organic matter found in drinking water. It is estimated that 180 million people in the United States drink disinfected water potentially containing DCA and TCA. Both compounds are also metabolites of trichloroethylene and tetrachloroethylene which have been identified as drinking water contaminants. DCA is used therapeutically for the treatment of various verrucae including calluses, corns and ingrown toenails. Other medical applications include its use in the treatment of lactic acidosis. TCA is also an ingredient used in pharmaceuticals. It is used as a cauterizing agent in medicine and dentistry, in the treatment of*

¹The information contained in this Executive Summary of Safety and Toxicity Information (ESSTI) is based on data from current published literature. The summary represents information provided in selected sources and is not claimed to be exhaustive.

warts, and for recurrent erosion of bullous keratopathy of the cornea. TCA has been used as an herbicide and has been reported to be an effective soil sterilant for perennial weed grass control. One manufacturer for DCA was listed in the public file of the EPA Toxic Substances Control Act (TSCA) Inventory, but no production data were available for. In addition, no production data for DCA were available from SRI's Chemical Economics Handbook. DCA was not listed in the United States International Trade Commission's (USITC) publication Synthetic Organic Chemicals. The total production volume of TCA was reported in the EPA TSCA Inventory in 1983 by four manufacturers to range from 1,101,000-11,110,000 pounds. No production data on TCA were available from the United States International Trade Commissions (USITC) publication Synthetic Organic Chemicals or from SRI's Chemical Economics Handbook.

Data from the National Occupational Exposure Survey (NOES) conducted during 1981-1983, estimated that 1,592 workers (579 females) were potentially exposed to DCA and 35,125 workers (16,821 females) were potentially exposed to TCA. DCA and TCA have been found to be present in the environment (ground and surface water, air, and soil) as a result of water disinfection, waste disposal, and the use of TCA as an herbicide. OSHA has not established a permissible exposure limit (PEL) for DCA ; however, the current OSHA PEL for TCA is 1 ppm (7 mg/m³). The ACGIH has not recommended a threshold limit value (TLV) for DCA, but has recommended a threshold limit value-time weighted average (TLV-TWA) for TCA of 1 ppm (6.7 mg/m³). NIOSH has not recommended an exposure limit (REL) for either compound.

Toxicological Effects

Human: DCA and TCA are corrosives and may result in moderate to severe skin and eye irritation in humans. In humans, TCA has been found to be primarily excreted through the kidneys; the half-life of TCA was determined to be approximately 50 hours. In vitro TCA was not found to induce peroxisome proliferation in cultured human hepatocytes. No data were found on the acute, prechronic, chronic, carcinogenic, reproductive, teratogenic, or mutagenic effects of DCA or TCA in humans.

Animal: The metabolism of TCA has been extensively studied in rats, mice, and dogs. In rats, it was determined that as much as 10% of the administered dose of TCA is excreted as carbon dioxide when administered orally or intravenously. The majority of TCA was excreted in the urine (58.7% of administered dose) within the first 24 hours. It was also detected in bile (10% of administered dose) and feces (1% of administered dose). In mice administered TCA intravenously, 15% of the administered dose was excreted as carbon dioxide; approximately 34% was excreted in the urine within 24 hours. No evidence of TCA was found in the feces. TCA was found to be only slightly metabolized in the extrahepatic organs of dogs (with extrahepatic bypass and without) administered intravenously. Only small amounts of TCA were excreted in the bile (approximately 1% after 2 hours) with 99% of the administered dose being excreted in urine. In dogs, TCA was slightly metabolized to free-trichloroethanol and conjugated trichloroethanol in the extrahepatic organs, liver, urinary bladder, gall bladder, and the ileal, colonic, and jejunal loops of the gastrointestinal tract. No data were found on the metabolism of DCA.

The acute oral LD_{50} of DCA was found to be 5520 mg/kg in mice and to range from 2820-4480 mg/kg in rats. DCA was found to be moderately to severely irritating to the skin and severely irritating to the eyes of rabbits. The oral LD_{50} for TCA was reported to be 4970 mg/kg in mice and to range from 3200-5000 mg/kg in rats. TCA was found to be a mild dermal irritant and a severe ocular irritant in rabbits. TCA was found to have a significant effect on body weight in rats in a 14-day study. In prechronic studies, rats exposed to DCA and TCA in drinking water were observed to have indications of systemic toxicity in the liver and kidney which included increases in organ to body weight ratios in the liver and kidney and focal areas of hepatocellular enlargement. However, in another 90-day dosed-water study, TCA was found only to have minimal toxic effects. TCA administered dermally in rats and mice in a prechronic study, did not produce any toxic effects.

Several studies conducted recently have confirmed, according to their authors, the hepatocarcinogenicity of DCA and TCA in mice exposed to the compounds in their drinking water. In one study, DCA and TCA were found to induce the formation of adenomas and hepatocellular carcinomas in mice in the presence and absence of an initiator (ethylnitrosourea) and were concluded to be complete carcinogens. Although TCA was not found to be carcinogenic in rats, the results of one study indicate that TCA may possess weak promoting activity in the rat. Dermal applications of TCA in mice increased the incidence of urethane-induced tumors.

Fetuses of rats that were orally administered DCA on days 6-15 of gestation had dose-dependent reductions in weight and length. Of the fetal malformations observed, cardiovascular malformations were the most prevalent; skeletal malformations were also observed, and DCA was determined to be teratogenic. In addition, TCA was found to cause embryonic death and fetal malformations (cardiovascular and skeletal systems) following exposure to rats early in organogenesis.

In one study reported, neither DCA nor TCA were observed to have immunotoxic effects. DCA was found to be a strong inducer of peroxisome proliferation (PP) when given to rats, but TCA was not observed to have this effect. However, in mice given these compounds orally, TCA was found to be a more potent inducer of PP than DCA. TCA was also observed to significantly increase ornithine decarboxylase (ODC) activity in rats; DCA had no significant effect on ODC activity in mice. DCA and TCA were both determined to be capable of causing single strand breaks (SSB) in rat and mouse DNA. DCA was found to be a more potent inducer of SSBs in rats and TCA was more potent in mice. However, one study indicated that neither of these compounds is capable of producing SSB's.

Genetic Toxicology: DCA and TCA have been found to be nonmutagenic in the Ames assay in all strains of Salmonella tested. Recently, an unpublished study by NTP found that DCA was mutagenic to Salmonella strains TA100 and TA1535. Both of these compounds were genotoxic in Salmonella TA1535/pSK1002 using the umu-test. Neither compound was found to be mutagenic in Klebsiella pneumoniae and TCA was not found to be mutagenic in Streptomyces coelicor, T4 bacteriophage, or Aspergillus nidulans. TCA was found to be capable of causing cytogenic mutations in mouse somatic and germ cells. TCA also produced mutations in plants (Arabidopsis and Faba vulgaris).

Structure Activity: Dichloroacetate and trichloroacetate have been found to cause hepatic tumors in mice following administration in the drinking water. In a study in which monochloroacetic acid was administered to rats and mice by gavage, no evidence of carcinogenic activity was observed.

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I. NOMINATION HISTORY AND REVIEW

A. Nomination History

1. Source: United States Environmental Protection Agency (USEPA) [USEPA, 1988]
2. Date: February, 1988
3. Recommendations: Carcinogenicity
4. Priority: High
5. Rationale/Remarks:
 - Breakdown product of drinking water disinfectants
 - Potential for extensive human exposure
 - Suspicion of carcinogenicity
 - NTP gavage carcinogenicity studies of monochloroacetic acid

B. Chemical Evaluation Committee Review

1. Date of Review:
2. Recommendation:
3. Priority:
4. NTP Chemical Selection Principle(s):
5. Rationale/Remarks:

C. Board of Scientific Counselors Review

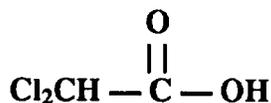
1. Date of Review:
2. Recommendations:
3. Priority:
4. Rationale/Remarks:

D. Executive Committee Review

1. Date of Review:
2. Decision:

II. CHEMICAL AND PHYSICAL DATA

A. Chemical Identifiers



DICHLOROACETIC ACID

CAS No. 79-43-6
RTECS No. AG6125000

Molecular formula: CHCl_2COOH

Molecular weight: 128.95

B. Synonyms and Trade Names

Synonyms: acetic acid, dichloro- (8CI) (9CI); bichloroacetic acid; DCA; dichloroacetic acid; dichlorethanoic acid; dichloroacetic acid; 2,2-dichloroacetic acid

Trade Names: Urner's liquid®

C. Chemical and Physical Properties

Description: DCA is a clear, colorless liquid [Sax and Lewis, 1987; Drinking Water and Health, 1987] with a pungent odor [Budavari, 1989; Drinking Water and Health, 1987].

Melting Point: 9.0-11.0°C (48.2-51.8°F) [Aldrich, 1990; Lenga, 1988]
9.7°C (49.5°F) or -4.0°C (24.8°F)²[Budavari, 1989; Dean, 1985]
13.5°C (56.3°F) [Weast, 1989]

Boiling Point: 193.0-194.0°C (379.4-381.2°F) [Budavari, 1989; Drinking Water and Health, 1987; Dean, 1985]
194.0°C (381.2°F) [Aldrich, 1990; Lenga, 1988; Weast, 1989]

Density 1.563 @ 20/4°C [Aldrich, 1990; Budavari, 1989; Lenga, 1988; Dean, 1985]
1.5634 [Weast, 1989]
1.564 [Kirk-Othmer, 1985]

²Melting point differs depending on the crystalline form of the compound

Refractive Index: 1.4659 @ 20°C [Budavari, 1989]
1.466 @ 20°C [Lenga, 1988; Aldrich, 1990]
1.4642 @ 20°C [Dean, 1985]
1.4658 @ 20°C [Weast, 1989]

Solubility in Water: Soluble in water [Budavari, 1989; Drinking Water and Health, 1987; Sax and Lewis, 1987; Weast, 1989; Dean, 1985].

Solubility in other Solvents: Soluble in alcohol, ether [Budavari, 1989; Weast, 1989; Dean, 1985], and acetone [Weast, 1989]

Log Octanol/Water Partition Coefficient: 0.73 [Poitras *et al.*, 1988]

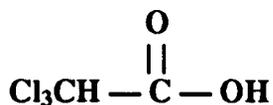
Reactive Chemical Hazards: Incompatible with strong oxidizing agents, strong bases, and strong reducing agents. Decomposition of DCA produces toxic fumes of carbon monoxide, carbon dioxide, and hydrogen chloride gas [Lenga, 1988]. Reacts with water or steam to produce toxic and corrosive fumes [Sax and Lewis, 1989].

Flammability Hazards:

- Combustible
- Flash point: > 110°C (230°F) [Aldrich, 1990; Lenga, 1988]
> 112°C (233.6°F) [Dean, 1985]
- Vapor pressure: 1 mm Hg @ 44.0°C [Sax and Lewis, 1989]
- Vapor density: 4.45 [Sax and Lewis, 1989]

A. Chemical Identifiers

TRICHLOROACETIC ACID



CAS No. 76-03-9
RTECS No. AJ7875000

Molecular formula: CCl_3COOH

Molecular weight: 163.40

B. Synonyms and Trade Names

Synonyms: acetic acid, trichloro- (8CI) (9CI); aceto-caustin; TCA; trichloroacetic acid; trichloroethanoic acid

Trade Names: Amchem grass killer®; Dow sodium TCA inhibited®; Konesta®

C. Chemical and Physical Properties

Description: TCA is a solid which occurs as white, deliquescent colorless crystals with a characteristic pungent odor [HHC, 1983; Budavari, 1989; Drinking Water and Health, 1987; Sax and Lewis, 1987; ITI, 1988].

Melting Point: 57.0-58.0°C (134.6-136.4°F) [Budavari, 1989; OHMTADS, 1991; Dean, 1985; ITI, 1988]
54.0-56.0°C (129.2-132.8°F) [Aldrich, 1990; Lenga, 1988]
58.0°C (136.4°F) (*alpha*) [Weast, 1989]
49.6°C (121.3°F) (*beta*) [Weast, 1989]

Boiling Point: 197.5°C (387.5°F) [Weast, 1989; Drinking Water and Health, 1987; HHC, 1983]
196.0-197.0°C (384.8-386.6°F) [ACGIH, 1986; Budavari, 1989; Dean, 1985; OHMTADS, 1991]
196.0°C (384.8°F) [Aldrich, 1990; Lenga, 1988]

Specific Gravity: 1.629 @ 61/4°C [Budavari, 1989; OHMTADS, 1991; Dean, 1985]
1.6218 @ 64/4°C [Weast, 1989]
1.6013 [HHC, 1983]
1.62 @ 25/4°C [Weast, 1989]

Refractive Index: 1.4603 @ 61°C [Weast, 1989]

Solubility in

Solubility in Water:

Soluble in water [Weast, 1989; ITI, 1988] (1.2 kg in 1 L of water at 25°C) [Drinking Water and Health, 1987], 1 in 0.1 part water [Budavari, 1989], 1306 g/100 g [HHC, 1983]

Solubility in other Solvents:

Soluble in alcohol [Budavari, 1989; Dean, 1985; Weast, 1989], 2143 g/100 g methanol, 850 g/100 g acetone, 617 g/100 g [HHC, 1983] ether [Budavari, 1989; Dean, 1985; Weast, 1989; Sax and Lewis, 1987; ITI, 1988], 201 g/100 g benzene, 106 g/100 g carbon tetrachloride, and 74 g/100 g *n*-heptane [HHC, 1983].

Log Octanol/Water Partition Coefficient:

1.17 [Poittrast *et al.*, 1988]

Reactive Chemical Hazards:

TCA is incompatible with strong oxidizing agents and strong bases [Lenga, 1988]. It can be decomposed by heating with caustic alkalies [Budavari, 1989]. When heated to decomposition it emits toxic fumes of Cl⁻ and Na₂O [Sax and Lewis, 1980]. Other decomposition products include: chloroform, alkali carbonate, hydrochloric acid [Budavari, 1989], carbon dioxide, carbon monoxide [Budavari, 1989; Lenga, 1988] and hydrogen chloride gas [Lenga, 1988].

Flammability Hazards:

- Nonflammable [HHC, 1983]
- Flash point: >110.0°C (230.0°F) [Aldrich, 1990; Lenga, 1988]
- Vapor pressure: 1 mm Hg @ 51.0°C [Sax and Lewis, 1989; Lenga, 1988]
5 mm Hg @ 76.99°C [HHC, 1983]
10 mm Hg @ 88.67°C [HHC, 1983]
20 mm Hg @ 101.52°C [HHC, 1983]
30 mm Hg @ 109.65°C [HHC, 1983]
40 mm Hg @ 115.72°C [HHC, 1983]
60 mm Hg @ 124.75°C [HHC, 1983]
100 mm Hg @ 136.98°C [HHC, 1983]
200 mm Hg @ 155.30°C [HHC, 1983]
400 mm Hg @ 175.96°C [HHC, 1983]
760 mm Hg @ 197.97°C [HHC, 1983]

III. PRODUCTION/USE

A. Production

1. Manufacturing Process

Dichloroacetic acid:

DCA has been prepared by the following manufacturing processes: the reaction of chloral hydrate with calcium carbonate (or sodium carbonate [Kirk-Othmer, 1979]) and sodium cyanide [Sax and Lewis, 1987; Kirk-Othmer, 1979] followed by acidification (88-92%); the chlorination of acetic acid (in the presence of iodine) [Sax and Lewis, 1987] and chloroacetic acid; electrolytic reduction of TCA; and the hydrolysis of pentachloroethane. DCA can also be separated from chloroacetic acid by the addition of azeotrope-forming hydrocarbons such as bromobenzene [Kirk-Othmer, 1978]. In addition, DCA has been prepared by treating TCA or aniline trichloroacetate with hydrochloric acid in the presence of copper powder [Kirk-Othmer, 1979].

Trichloroacetic acid:

TCA is produced at a purity of 95% from the exhaustive chlorination of acetic acid [Kirk-Othmer, 1978]. In addition, TCA can be derived from the treatment of chloral hydrate with fuming nitric acid [Budavari, 1989; Sax and Lewis, 1987; Kirk-Othmer, 1978], the breakdown of glacial acetic acid by the action of chlorine in the presence of sunlight, ultraviolet radiation, or catalysts [Sax and Lewis, 1987], or via hydrolytic oxidation of tetrachloroethene [Kirk-Othmer, 1978].

2. Producers and Importers

U.S. Producers

Dichloroacetic Acid

Producers

American Hoechst Corporation
Bridgewater, New Jersey

Eastern Chemical
Division United-Guardian, Incorporated
Smithtown, New York

Hoechst Celanese Corporation
Fine Chemicals Division
Dallas, Texas

Reference

USEPA, 1991a

OPD Chemical Buyers
Directory, 1990

OPD Chemical Buyers
Directory, 1990

Producers (cont.)

3M Company
Decatur, Alabama

Pfizer, Incorporated
New York, New York

Rhone-Poulenc, Incorporated
Freeport, Texas

White Chemical Corporation
Newark, New Jersey

Reference (cont.)

USEPA, 1991a

USITC, 1989

USEPA, 1991a

SRI, 1990a

Trichloroacetic Acid

Producers

Amend Drug and Chemical Company,
Incorporated
Irvington, New Jersey

American Hoechst Corporation
Bridgewater, New Jersey
North Hollywood, California

Columbus Chemical Industries, Incorporated
Columbus, Wisconsin

Dow Chemical Company
Midland, Michigan

Haven Chemical
Philadelphia, Pennsylvania

Hoechst Celanese Corporation
Fine Chemicals Division
Charlotte, North Carolina

3M Company
Decatur, Alabama; Cordova, Illinois

Montsanto Agricultural Company
St. Louis, Missouri

Reference

OPD Chemical Buyers
Directory, 1990

OHMTADS, 1991
USEPA, 1991a

OPD Chemical Buyers
Directory, 1990

OHMTADS, 1991
USEPA, 1991a

USEPA, 1991a

OPD Chemical Buyers
Directory, 1990

USEPA, 1991a

USITC, 1989

Rhone-Poulenc, Incorporated
Freeport, Texas

USEPA, 1991a

Roussel Corporation
New York, New York

USEPA, 1991a
OPD Chemical Buyers
Directory, 1990

Ruger Chemical Corporation, Incorporated
Irvington, New Jersey

OPD Chemical Buyers
Directory, 1990

Spectrum Chemical Manufacturing Corporation
Gardena, California

OPD Chemical Buyers
Directory, 1990

European Producers

Dichloroacetic Acid

Producers

Reference

Hoechst AG
Frankfurt, Germany

SRI, 1990b

Trichloroacetic Acid

Producers

Reference

BASF Aktiengesellschaft
Ludwigshafen, Germany

SRI, 1990b

Bayer AG
Leverkusen, Germany

SRI, 1990b

Hoechst AG
Frankfurt, Germany

SRI, 1990b

Dichloroacetic Acid

Importers

Reference

American Hoechst Corporation
Bridgewater, New Jersey

USEPA, 1991a

Rhone-Poulenc, Incorporated
Freeport, Texas

USEPA, 1991a

Trichloroacetic Acid

Importers

American Hoechst Corporation
North Hollywood, California

Rhone-Poulenc, Incorporated
Freeport, Texas

Roussel Corporation
New York, New York

Reference

OHMTADS, 1991
USEPA, 1991a

USEPA, 1991a

USEPA, 1991a

3. Volume

Dichloroacetic acid:

Production Volume

DCA is not listed in the United States International Trade Commission's (USITC) publication Synthetic Organic Chemicals and no production data were available on DCA from this source for the years 1985-1988 [USITC, 1986-1989].

One company is listed as a manufacturer of DCA in the public file of the EPA Toxic Substances Control Act (TSCA) Inventory, but no information was provided on volume [USEPA, 1991]. No production data for DCA were included in SRI's Chemical Economics Handbook [SRI, 1991c].

Import Volume

Two companies are listed as importers of DCA in the EPA TSCA Inventory, but no import volumes were reported [USEPA, 1991a].

Trichloroacetic acid:

Production Volume

TCA is listed in the United States International Trade Commission's (USITC) publication Synthetic Organic Chemicals. However, no production data were available on TCA from this source for the years 1985-1988³ [USITC, 1986-1989].

The volume of TCA manufactured in the United States is reported in the public file of the EPA Toxic Substances Control Act (TSCA) Inventory. Four companies listed as manufacturers reported a total volume ranging from 1,101,000 - 11,110,000 pounds. One of the manufacturing companies lists two plants, one of which is classified as a small manufacturer (less than 100,000 pounds of TCA were produced). Two additional manufacturers were listed, but production volumes were not reported [USEPA, 1991a].

No production data for TCA were found in SRI's Chemical Economics Handbook, however it was estimated that in 1986, 2% of monochloroacetic consumed in Western Europe was used for TCA production [SRI, 1991c].

Import Volume

Three companies are listed as importers of TCA in the EPA TSCA Inventory, but no import volumes were reported [USEPA, 1991a]. The U.S. Imports for

4. Technical Product Composition

Dichloroacetic Acid

DCA can be purchased at a purity of >99% [Aldrich, 1990].

Trichloroacetic Acid

TCA is available as technical, C.P., and U.S.P. grades [NSC, date unspecified]. It can be purchased at purities of 98 and >99% [Aldrich, 1990].

³Production statistics for an individual chemical are given only when there are three or more producers, no one or two of which may be predominant. Moreover, even when there are three or more producers, statistics are not given if there is any possibility that the publications would violate the statutory provisions relating to unlawful disclosure of information accepted in confidence by the Commission. Data are reported by producers for only those items where the volume of production or sales exceeds certain minimums. Those minimums for all sections are 5,000 pounds of production or sales, or \$5,000 value or sales with the following exceptions: plastics and resin material — 50,000 pounds or \$50,000; pigments, medicinal chemicals, flavor and perfume materials, and rubber processing chemicals — 1,000 pounds or \$1,000.

B. Use

Dichloroacetic Acid

DCA is used as a topical astringent and keratolytic [Curry, 1989]. DCA rapidly penetrates and cauterizes skin, keratin, and other tissues. Its cauterizing effect is comparable to that obtained with such methods as electrocautery or freezing. This compound can be used to treat all types of verrucae including calluses, hard and soft corns, xanthoma palpebrarum, seborrheic keratoses, ingrown toenails, cysts, and benign erosion of the cervix (endocervicitis and epistaxis) [PDR, 1991]. Other medical applications for DCA include its use in the treatment of lactic acidosis [Budavari, 1989]. DCA is also employed in the production of organic [Sax and Lewis, 1987] and pharmaceutical products [Sax and Lewis, 1987; ITI, 1989].

Trichloroacetic Acid

TCA is commonly used as a facial chemical peel [Brodland and Roenigk, 1988; Lober, 1987; Collins, 1989] in the treatment of actinic keratoses (pre-malignant precursors of squamous cell and basal cell carcinomas) [Brodland and Roenigk, 1988]. TCA is also frequently used for the treatment of aging, wrinkled, and sun-dried skin [Collins, 1989]. Other therapeutic applications for TCA include the following:

- Chemical cauterizing agent in medicine and dentistry since the 19th century [Heithersay and Wilson, 1988]
- Component of aqueous solutions (10-25%) used to treat recurrent erosion and bullous keratopathy of the cornea [Grant, 1986]
- Reagent for detection of albumin [ACGIH, 1986; Drinking Water and Health, 1987; Sax and Lewis, 1987; ITI, 1988]
- Removal of warts [Drinking Water and Health, 1987]
- Astringent [Drinking Water and Health, 1987]

TCA is used as a component in organic synthesis [Drinking Water and Health, 1987; Sax and Lewis, 1987; ITI, 1988]. In addition, TCA has been used as an herbicide [OHMTADS, 1991; Sax and Lewis, 1987; Drinking Water and Health, 1987; Plunkett, 1987; Sax and Lewis, 1986]. It is an effective soil sterilant (inhibits seed germination and plant growth for at least 60 days) which has been used for perennial weed grass control on non-cropland and stubble. TCA can be used selectively to control seedling grasses and some broadleaf weeds in sugar beets, sugar cane, and oilseed rape [Meister, 1987]. However, the EPA reports (personnel communication) that TCA has been banned for use as an herbicide in the United States [USEPA, 1991b; USEPA, 1991c].

IV. EXPOSURE/REGULATORY STATUS

A. Consumer Exposure

DCA and TCA are by-products formed during the chlorination of water containing natural organic matter (primarily humic substances) [Kopfler, 1985; Christman *et al.*, 1983; Reckhow, *et al.*, 1990; Voogd *et al.*, 1989; Smith *et al.*, 1989a; Smith *et al.*, 1988]. It is estimated that 180 million people in the United States drink disinfected water that potentially contains DCA and TCA. The EPA Office of Drinking Water reports that DCA and TCA occur in the 10-100 µg/L range in drinking water [USEPA, 1988].

In addition, trichloroethylene, which is metabolized to TCA and DCA, has been found in drinking water at concentrations ranging from 0-500 ng/L. Thus, consumer exposure to DCA and TCA may result indirectly from the ingestion of drinking water containing trichloroethylene [Herren-Freund *et al.*, 1987a].

In an Italian study, 141 blood donors were analyzed for plasma and urinary levels of TCA and trichloroethanol (TCEH) from exposure to different levels of trichloroethylene (TCE) and tetrachloroethylene (PER) in drinking water or in the air. More than 40% of subjects studied had TCEH/TCA ratios between 1 and 2. The authors state that the presence of TCA indicates that both TCE and PER are taken up and metabolized to TCA. TCA levels in individuals exposed to TCE and PER in drinking water are statistically higher than in those who do not drink water contaminated with these compounds, although some individuals not exposed to TCE and PER have been found to have levels of TCA in their plasma and urine. TCA levels found in test subjects did not appear to be related to sex, age, or body size [Ziglio *et al.*, 1985].

B. Occupational Exposure

Dichloroacetic Acid

Data from the National Occupational Exposure Survey (NOES), conducted by the National Institute for Occupational Safety and Health (NIOSH) during the years 1981 to 1983, estimated that 1,592 workers, including 579 females, were potentially exposed to DCA. Of the workers potentially exposed to DCA, 1,505 (514 females) were chemists (except biochemists), and 87 were (65 females) chemical technicians, the data were obtained from 61 locations [NIOSH, 1991].

Trichloroacetic Acid

Data from the National Occupational Exposure Survey (NOES), conducted by the National Institute for Occupational Safety and Health (NIOSH) during years 1981 to 1983, estimated that 35,125 workers, including 16,821 females, were potentially exposed to TCA. This data was obtained from 1,562 locations. The highest number of potential exposures was reported in the following industries: oil and gas extraction, chemicals and allied products, business services, and health services. Also, the occupations that had more than 1,000 employees potentially exposed included the following: physicists and astronomers; chemists (except biochemists); clinical laboratory technologists and technicians; health technologists and technicians; engineering, biological, and chemical technicians; separating, filtering, and clarifying machine operators; mining machine operators; and health aides (except nursing). The NOES data base does not contain information on the frequency, level or duration of exposure to workers of any chemicals listed therein [NIOSH, 1991].

C. Environmental Occurrence

- The formation of DCA and TCA from water chlorination is well documented [Kopfler *et al.*, 1985; Christman *et al.*, 1983; Johnson *et al.*, 1982; Reckhow, *et al.*, 1990; Voogd *et al.*, 1989; Norwood *et al.*, 1985]. These by-products can arise in the environment from a reaction between chlorine and humic acids [Kopfler *et al.*, 1985; Christman *et al.*, 1983; Reckhow, *et al.*, 1990; Voogd *et al.*, 1989] and fulvic acids [Reckhow, *et al.*, 1990]. In a study conducted by the EPA to develop a priority list of chemicals most often found in water, it was determined that haloacetic acids, including DCA and TCA, were the second largest class (on a weight basis) of disinfection by-products (DBPs) detected [Krasner *et al.*, 1989].

The following studies have been carried out to investigate the environmental occurrence of DCA and TCA:

- A study was conducted to determine the effectiveness of aerating lagoons in reducing the amount of chlorinated organics, including DCA and TCA, occurring in kraft pulp mill effluents in Sweden. The influent and effluent waters were tested and compared for levels of chlorinated organics. DCA was found at an average concentration of 1290 µg/L and 93 µg/L in the influent and effluent, respectively; TCA was determined to be present at an average concentration of 2500 µg/L and 865 µg/L in the influent and effluent, respectively. It was determined that DCA and TCA can be efficiently removed in lagoons aerated for 2.5-5.0 days [Lindström and Mohamed, 1988].

- DCA and TCA were identified in biologically treated wastewater effluent from the SFI Pulp and Paper Mill in Malaysia at concentrations of 14-18 µg/L and 838-994 µg/L, respectively. It was found that oxygen activated sludge treatment was 30-99% effective in removing chlorinated organics such as DCA and TCA from the wastewater. Of the compounds, DCA was found to be more biodegradable, although it was determined that it could be efficiently removed by prolonged retention during treatment [Mohamed *et al.*, 1989].
- DCA and TCA are ubiquitous air pollutants in Southern Germany that have been blamed for deforestation in relatively nonpolluted areas. These compounds have been found in spruce needles at concentrations ranging from ten to several hundred µg/kg. The authors report that there is some correlation between the levels of DCA and TCA in the needles and the loss of foliage. The absolute atmospheric values of DCA and TCA have been observed to change with meteorological conditions. For example, during sunny and dry periods levels of these compounds may be high, but will decrease during rainy periods [Frank *et al.*, 1989].

The following studies were carried out to investigate the environmental occurrence of DCA:

- DCA was one of over 60 compounds identified in the groundwater beneath the Savannah River Plant's waste burial ground. This burial site was used by the Savannah River plant between 1953 and 1972 to dispose of solid, low-level radioactive waste [Oblath, 1987].
- DCA was one of approximately 80 compounds that has been identified in the spent chlorination liquor from the bleaching of sulphite pulp. DCA was detected by gas chromatography/mass spectrometry at concentrations of 0.2-0.5 g/ton pulp [Carlberg *et al.*, 1986].

The following studies were carried out to assess the environmental occurrence of TCA:

- A study was conducted to determine if a difference in disinfection by-products exists among different types of raw water sources. Finished water samples from the three different types of raw water sources (rivers, impoundments, and chlorinated groundwater) were collected and analyzed for TCA. TCA was detected in every sample tested at concentrations ranging from 4.23-53.8 µg/L. The authors concluded that TCA is an important and possibly ubiquitous by-product of water disinfection with chlorine [Norwood *et al.*, 1985].
- Sampling of elution waters in the Murrumbidgee Irrigation Areas and Districts of Australia revealed high levels of TCA 6 weeks after spraying the banks with this herbicide. Following this discovery, studies were conducted to determine the rate of TCA dissipation from sediments on the banks of the canal. It was concluded that TCA was not fully dissipated because of a slow final phase; therefore, the length of time between recharging the canals and spraying was extended [Bowmer, 1987].

- TCA has been found in the irrigation waters of 4 canals in Washington state. The presence of TCA was attributed to the spraying of herbicides containing TCA, as a sodium salt formulation, on the dry banks of the canals. The maximum concentrations of TCA were detected in the irrigation water (ranging from 53 to 297 ppb) while the canals were being recharged, but only for the first 4 hours of the recharging process. After the first 4 hours, the levels of TCA decreased, and this compound was no longer detected after all the "left over" herbicide residues had been washed away [Comes *et al.*, 1975].
- In a Japanese study, it was determined that TCA residues can remain in soil (under field conditions) for 7 months and that TCA is absorbed by crops. The amount of TCA absorbed by corn and wheat from the soil was measured at different stages of growth. It was found that the concentrations of TCA rapidly decreased after the trifoliolate stage [Qiong *et al.*, 1989].
- TCA has been used at high doses (20-30 kg/ha) as an herbicide and is considered to be highly mobile in soil. Because of these factors, a study was conducted to determine the degradation capacity of TCA at different levels in soil samples from Sweden to estimate the likelihood of its reaching groundwater. Soil samples from five different locations and depths were analyzed for respiration, microbial growth, the relative degradation capacity of TCA, and organic content.

It was found that TCA is primarily degraded in the soil by a microbial process in which microorganisms use TCA as a source of energy. Microorganisms were found in the greatest number near the soil surface (0-5 cm). Other factors having a significant influence on TCA degradation were the content of organic matter, biological activity of the soil (respiration rate), and pH. Many of the conditions required for optimal degradation exist between 0-5 cm in the soil where degradation occurs most rapidly (50% degradation in 2-9 weeks). However, the study showed that there is some evidence of degradation at 95-100 cm, indicating that TCA is capable of travelling beyond the surface and contaminating the groundwater [Torstensson and Hammarström, date not specified].

- In Wexford, Ireland, the influence of climatic and microbial factors on the persistence of TCA in varying types of soils was studied. Soil samples from three locations within Wexford were collected for analysis. It was determined that microorganisms in the soil, and not the soil composition, were responsible for the degradation of TCA. Likewise, the persistence of TCA in soil can be influenced by its application during previous years. Persistence is also influenced by the climate at the time of spraying. For instance, spring-applied herbicide is likely to be less persistent than an application of herbicide in the fall. The authors state that if the temperatures were higher and there was less rainfall, the observed effects may be more pronounced [McGrath, 1976].

- In a Danish study, the adsorption and phytotoxicity of TCA in soils were examined in relation to the different properties of soil samples. Nine soil samples were analyzed for exchangeable calcium (Ca), pH, cation exchange capacity, organic matter, clay, and field water capacity (FC). TCA exhibited little or no adsorption to soil, and in some cases, negative adsorption occurred. Selection of FC, which exerts a dilutory effect on TCA concentration in soil water, supports the assumption that adsorption to soil is negligible. Phytotoxicity of TCA was correlated with several soil properties including exchangeable Ca, FC, and organic content. It was established that the "best" predictors of phytotoxicity were increasing acidity and FC which decreased the phytotoxicity of TCA [Streibig, 1980].
- The mobility (R_F) of TCA on Hagerstown silty clay loam plates was found to be 0.96 using thin-layer chromatography. Based on this R_f value, TCA was assigned to mobility class 5, a class includes the most mobile of the 40 compounds tested in this study [Helling, 1971].

D. Regulatory Status

- The United States Environmental Protection Agency is currently developing regulations to control disinfection by-products, including TCA and DCA, as a result of the 1986 amendments to the Safe Drinking Water Act. Under these amendments, the USEPA is required to develop a priority list of chemicals. The proposed date for this list is 1993 and the projected finalization date is 1995 [USEPA, 1991b].

Dichloroacetic Acid

- OSHA has not established a permissible exposure limit (PEL) for DCA.

Trichloroacetic Acid

- The current Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for TCA is 1 ppm (7 mg/m³) {21 CFR Part 1910} [Office of the Federal Register, 1989].
- TCA is regulated under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and is currently banned from use as an herbicide in the U.S. [USEPA, 1991c].

E. Exposure Recommendations

Dichloroacetic Acid

- ACGIH has not recommended a threshold limit value (TLV) for DCA.
- NIOSH has not recommended an exposure limit (REL) for DCA.

Trichloroacetic Acid

- The ACGIH-recommended threshold limit value-time weighted average (TLV-TWA) for TCA is 1 ppm (6.7 mg/m³) [ACGIH, 1986; ACGIH, 1990].
- NIOSH has not recommended an exposure limit for TCA.

V. TOXICOLOGICAL EFFECTS

A. Chemical Disposition

1. Human Data

a) Dichloroacetic Acid

No data were found.

b) Trichloroacetic Acid

- ***oral, human:***

In a study to determine the pharmacokinetics of TCA, 3 healthy male volunteers (20-30 years of age) ingested 3 mg/kg sodium-trichloroacetate dissolved in water. Drug and alcohol intake was discontinued for several days before and during the study. The concentrations of TCA in the plasma and urine were determined.

Peak levels of TCA in both the plasma and urine (approximately 30 µg/ml and 40 mg, respectively) were observed immediately following dosing, but the levels decreased over time. The amount of TCA recovered from urine was about 24%. The authors state that only half of the administered TCA is recovered in urine. Excretion of TCA is through the kidneys which may indicate further metabolism. Furthermore, TCA was determined to have a half-life of about 50 hours [Müller *et al.*, 1974].

2. Animal Data

a) Dichloroacetic Acid

No data were found.

b) Trichloroacetic Acid

• oral rats :

The metabolism of TCA was studied in rats to better understand the metabolism of trichloroethylene (TCE). One male Osborne-Mendel rat was given a single 75 mg/kg oral dose of TCA radiolabeled on the second carbon position ([2-¹⁴C]TCA, 9 µCi) as a solution in water (5 ml/kg). Urine, feces, and expired air were collected for 24 hours after dosing. Hexane and 2N sodium hydroxide traps were used to capture the expired air from the test animals, which was analyzed using gas chromatography (GC) fitted with both flame ionization and radiochemical detectors. Radiolabeled mass peaks were identified by gas chromatography-mass spectroscopy (GC-MS). Urine samples were collected, acidified, and extracted. The extracts were then concentrated and analyzed using GC and GC-MS. The residual urine was concentrated and chromatographed, fractions were collected, and the radioactivity was determined using liquid scintillation counting. The fractions containing radioactivity were evaporated to dryness, and the trimethylsilyl derivatives were prepared with BSTFA in pyridine. The derived fractions were analyzed by GC and GC-MS. In an additional experiment, two rats (of an unspecified strain) were surgically equipped with a biliary cannula and given a single oral dose (100 mg/kg) of radiolabeled TCA ([2-¹⁴C]TCA; 15.5 µCi) as a solution in water (5 ml/kg). The animals were contained in restraining cages and bile was collected for 24 hours. The bile samples were analyzed both quantitatively and qualitatively by the same methods used to analyze the urine.

Results indicated that 7.2% of the [2-¹⁴C]TCA was excreted as carbon-14 labelled carbon dioxide [¹⁴CO₂] in 24 hours. Over the same period, 58.7% and 1% of the dose was excreted in the urine and feces, respectively. Difficulties with the insertion of the cannula made collection of bile difficult. However, 10% of the [2-¹⁴C]TCA was excreted in the bile during the first 24 hours. The authors state that one of the most significant findings in this study was the discovery that the CO₂ from TCE was not from trichloroethylene oxide (as formally believed) but rather from TCA. CO₂ was found to be a major metabolite of TCA (as much as 10% of a single dose). This suggests that further TCA metabolism is a significant, and possibly major source of TCE-derived CO₂ [Green and Prout, 1985].

- **oral, rats :**

The rate at which TCA is absorbed from the gastrointestinal tract into the circulatory system was determined by administering TCA-milk solutions to 21 day old Fisher (F-344) rat pups. The rat pups (3-6 animals per sacrifice period) were gavaged with 0.1 ml of TCA milk solution (5 mg/kg). The animals were sacrificed at 5 and 30 minutes, and at 1, 2.5, and 6 hours after the exposure; heart blood samples were collected from each animal.

The oral uptake rates (K_a) were determined from the blood samples taken. The appearance of TCA into the systemic circulation was described by a first order rate constant (K_a) of 1.0/hour. The peak TCA plasma concentration occurred at about 2.5 hours after gavage, which was followed by a slow elimination phase from the plasma [Fisher, 1987].

- **intraperitoneal, rats:**

In a study investigating the metabolic pathway of trichlorethylene (TCE) and its major metabolites, 14 male Holtzman rats were administered a 10 mg intraperitoneal dose of either TCA (N=3), TCE, or another metabolite. Urine samples were collected every 24 hours for 3 days after administration of the test compounds. The total amount of TCA and trichloro-compounds (sum of TCA, free trichloroethanol, and urochloralic acid) were measured, then total trichloroethanol (TCEH; free trichloroethanol, and urochloralic acid) was calculated as the difference between total trichloro-compounds (TTC) and TCA.

Following administration of TCA, urinary excretion of TCA, TCE, and TCEH was highest within the first 24 hours and rapidly decreased thereafter. By the fourth day of the study, the levels of TCA were undetectable. The urinary excretion of TCA and its metabolites was observed to be slower in rats administered TCA than in rats administered TRI or the other metabolites. The authors stated that the TCA is excreted into the urine unchanged [Nomiyama and Nomiyama, 1979].

The study described above also examined the metabolic rates of TCE and its major metabolites (including TCA). For this experiment, eight male Holtzman rats were administered a single intraperitoneal dose of 30 mg TCA in a 1.0 ml saline solution. The rats were further divided into four subgroups, and urine samples were collected from two rats at 5, 10, 20, or 30 minutes after administration of the test material. Urine samples were collected from all of the test animals 1, 2, 4, 6, 12, and 24 hours after dosing. Gas liquid chromatography was used to measure the concentrations of TCA in the urine.

Following administration of TCA, concentrations of TCA in the urine reached peak levels (approximately 10 mg) about 24 hours after dosing. These levels were found even after 48 hours. The authors stated that the excretion of TCA extended over a longer period of time due to its high plasma protein binding [Nomiyama and Nomiyama, 1979].

- **intraperitoneal rabbits:**

In a study examining the metabolic rate of trichloroethylene and its metabolites (see previous bullet), one Japanese white rabbit was given a 0.5 g intraperitoneal injection of TCA. Urine samples were collected every 12 hours for 4 days beginning 24 hours prior to dosing. Concentrations of TCA, total trichloro-compounds (TTC), and trichloroethanol (TCEH) were measured in each sample. After 4 days, the majority of the TCA administered was excreted into the urine as TCA (234.1 mg) or TTC (228.1 mg); only 4.5 mg of TCEH was measured in the samples. The maximum levels of TCA and TTC were observed within the first 12 hours after dosing. After this time, there was a slow decrease in urinary excretion of the two compounds [Nomiyama and Nomiyama, 1979].

- **intravenous rats:**

The volume of distribution (V_d) and elimination rate constants (K_e)⁴ of TCA were determined using nonpregnant, pregnant, and lactating Fisher (F-344) rats. Three unanesthetized nonpregnant rats were given an injection in the femoral vein of 0.1 ml of TCA-physiological saline, and blood samples were collected at 1, 4, 12, 24, 30, and 48 hours after injection. Four pregnant rats were administered 4.0 mg/kg TCA-saline solution, via femoral injection, on days 14-15 of gestation. Blood samples were collected from each of the animals at 1, 3, 10, 22, 28, and 47 hours after administration of the compound. Finally, 4 lactating rats were injected with a 4.4 mg/kg TCA-saline solution, via the femoral vein, on days 16-17 of lactation. Blood samples were collected at 1, 3, 12, and 23 hours post-exposure. For all samples, the plasma TCA elimination rates were calculated by plotting the \log_{10} values of the plasma TCA concentration data on a linear time scale. Linear regression analyses of the data were used to determine the slope (S) of the line.

⁴Volume of distribution was calculated as follows (C_o , is the initial TCA plasma concentration):

$$V_d = \frac{\text{dose (mg/kg)}}{C_o \text{ (mg/kg)}} \text{ (L/kg)}$$

K_e was calculated by the following equation: $K_e = S \times 2.303 \text{ (hour)}$

Following an intravenous dose of TCA, the elimination rates in the rats were dependant upon the state of the animal; overall elimination curve slopes were significantly different ($P < 0.0001$) in lactating, pregnant and nonpregnant rats. Further analysis, however, indicated that the TCA K_e values were not significantly different ($P > 0.05$) between nonpregnant (0.090/hour ($t_{1/2} = 7.8$ hour)) and lactating rats (0.086/hour ($t_{1/2} = 8.2$ hour)), whereas the K_e in pregnant rats was statistically slower ($P < 0.0001$), (0.045/hour ($t_{1/2} = 15.4$ hour)). The 95% confidence intervals for K_e 's in the nonpregnant, lactating, and pregnant rats were 0.085-0.096, 0.070-0.100, and 0.039-0.053, respectively.

The authors stated that TCA is not readily distributed in the body of nonpregnant, pregnant, and lactating rats. The determined V_d values for TCA in nonpregnant, pregnant, and lactating rats were 0.511, 0.610, and 0.541 L/kg, respectively [Fisher, 1987].

- **intravenous rats:**

The ability of TCA to cross the placental barrier into fetuses and cross the mammary tissue into milk was studied and quantitatively compared in pregnant and lactating Fisher (F-344) rats. The TCA levels were quantitatively compared in the dams, fetuses, and neonates by determining the cumulative area under the curve (AUC). Three pregnant rats (day 20 of pregnancy) were administered 0.1 ml of a TCA physiological saline solution (65 mg/kg TCA), and 3 lactating rats (day 13 of lactation) were given 0.1 ml of a TCA-saline mixture (8.0 mg/kg TCA) by injection into the femoral vein. The lactating rats were anesthetized and given an intraperitoneal injection of oxytocin (0.25 mg/kg body weight) 1, 2, and 4 hours after exposure to the TCA. Blood and milk samples were collected. The three pregnant rats were sacrificed 45 minutes⁵, and 2 and 4 hours postadministration of TCA. Samples of maternal and pooled fetal blood were collected for analysis.

The blood and milk samples were analyzed to determine the concentration of TCA present. The levels of TCA in fetal plasma and milk were lower than the levels found in the maternal plasma. The following TCA plasma levels were reported in the pregnant dams: 184 $\mu\text{g/mL}$ (45 minutes), 124 $\mu\text{g/mL}$ (2 hours), and 89 $\mu\text{g/mL}$ (4 hours) after TCA injection. Fetal TCA plasma levels were reported as 113 $\mu\text{g/mL}$ (45 minutes), 118 $\mu\text{g/mL}$, (2 hours) and 69 $\mu\text{g/mL}$ (4 hours). The concentrations of TCA in the plasma of the lactating animals were 23.2 $\mu\text{g/mL}$, 18.7 $\mu\text{g/mL}$, and 15.5 $\mu\text{g/mL}$ at 1, 2, and 4 hours after TCA exposure, respectively. The TCA concentrations in the milk were measured as 5.0 $\mu\text{g/mL}$, 4.7 $\mu\text{g/mL}$ and 3.2 $\mu\text{g/mL}$ after 1, 2, and 4 hours postinjection, respectively. The authors established that TCA is capable of penetrating the placental and mammary barriers as TCA was detected in fetal plasma and milk [Fisher, 1987].

⁵ In the description of the experiment it was reported that one of the pregnant dams was sacrificed 45 minutes post-exposure to TCA. However, the results are reported only for 15 minutes, 2- and 4-hours postinjection time points. In preparing this summary, it was assumed that the data presented in the study represent 45-minute, and 2- and 4-hour results.

- **intravenous, rats:**

The metabolism of TCA when administered intravenously to rats was studied to better understand the metabolism of trichloroethylene (TCE). This study was carried out in conjunction with a metabolism study described previously (Green and Prout, 1985) in which TCA was administered orally to rats. Four male Osborne-Mendel rats were injected in the tail vein with a 10 mg/kg dose of TCA radiolabeled in the C-2 position ($[2-^{14}\text{C}]\text{TCA}$, 12.5 μCi) as a solution in water (0.25 ml). The animals' expired air, urine, and feces were collected for 24 hours after the dosing. Hexane and 2N sodium hydroxide traps were used to capture the expired air from the test animals which was analyzed using gas chromatography (GC). Urine samples were collected, acidified and extracted. The extracts were then concentrated and analyzed using GC and GC-MS. The residual urine was concentrated and chromatographed. Four millimeter fractions were collected and the radioactivity was determined using liquid scintillation counting. The fractions containing radioactivity were evaporated to dryness and the trimethylsilyl derivatives were prepared with BSTFA in pyridine. The derived fractions were analyzed by GC and GC-MS.

In 24 hours, $12.3\pm 1\%$ of the $[2-^{14}\text{C}]\text{TCA}$ was excreted as carbon-14 labelled carbon dioxide (CO_2). Urinary excretion of radioactivity was $34.5\pm 10\%$. No results were reported concerning the amount of radioactivity detected in feces. The realization that CO_2 from TCE was not from trichloroethylene oxide (as formally believed) but rather from TCA was the most significant finding in this study. As much as 10% of a single dose of TCA was metabolized to CO_2 . This suggests that further TCA metabolism is a significant, and possibly the major source of TCE-derived CO_2 [Green and Prout, 1985].

- **intravenous, mice:**

The study described in the previous bullet was repeated using mice to compare species-related differences in TCA metabolism. Four male mice (of an unspecified strain) were treated in the same fashion as the rats described above with TCA radio-labeled in the C-2 position (10 mg/kg, 3.9 μCi), as a solution in water (0.1 ml).

In the first 24 hours following dosing, $15.3\pm 1\%$ of the $[2-^{14}\text{C}]\text{TCA}$ was excreted as carbon-14 labelled carbon dioxide in 24 hours. Urinary excretion of radioactivity was $34.7\pm 6\%$. No results were reported on the amount of radioactivity detected in feces. The authors did not find any significant differences in the metabolism of TCA between rats and mice [Green and Prout, 1985].

- ***intravenous dogs***

In a study examining the extrahepatic metabolism of TCA, two groups of 5 mongrel dogs were given an intravenous injection of 25 mg/kg TCA, via the left femoral vein. One of the groups underwent a surgical procedure to install an extrahepatic bypass; the non-bypass group served as the control group. Arterial blood and urine samples were collected prior to administration of TCA, and 30, 60, 90, and 120 minutes after administration of TCA. Urine samples were collected at five 30-minute intervals before dosing, and then at 30, 60, 90, and 120 minutes after dosing. All samples were analyzed to determine the levels of TCA and its metabolites present. In addition, biological and physiological parameters including heart rate and rhythm, blood pressure, erythrocyte and leukocyte counts, and blood chemistries (GOT, GPT, alkaline phosphatase, hematocrit, blood urea nitrogen, ammonia, and creatine) were evaluated.

There were no significant differences in the biological or physiological parameters between the bypass and non-bypass animals. However, TCA levels in the bypass group were found to be significantly ($P < 0.01$) higher than those in the non-bypass animals. The levels of TCA in the serum and urine of the bypass dogs were 130-150% and 230-270% those of the non-bypass group, respectively. In both test groups, the serum levels of TCA were observed to decrease over time, while urinary TCA levels abruptly increased. Table 1 summarizes the levels of TCA measured in the urine and serum of the test animals. In non-bypass dogs, 1.3% of the administered dose was excreted in the urine after 2 hours, while 3.3% was excreted in the urine of the bypass group. In both cases, 99.9% of administered TCA was excreted in the urine as TCA as opposed to a metabolite (such as chloral hydrate or trichloroethanol).

The authors conclude that TCA is only slightly metabolized in the extrahepatic organs, thus explaining the high levels remaining in the serum, and the high levels excreted in the urine as unchanged compound [Hobara *et al.*, 1987a].

Table 1: The Concentrations of TCA in the Serum and Urine Following Intravenous Administration in Dogs

		Concentration of TCA ($\mu\text{mol}/\text{ml}$) at the indicated time after administration			
<u>Specimen</u>	<u>Group</u>	<u>30 min</u>	<u>60 min</u>	<u>90 min</u>	<u>120 min</u>
Serum	C	659.4 \pm 93.7	539.4 \pm 95.1	470.9 \pm 80.2	458.8 \pm 61.3
	E	975.6 \pm 115.9	732.8 \pm 104.	641.8 \pm 93.5	573.2 \pm 72.6
Urine	C	538.1 \pm 62.0	886.2 \pm 106.3	1097.0 \pm 149.0	1526.0 \pm 277.0
	E	1254.0 \pm 1	2319.0 \pm 256.0	2937.0 \pm 315.0	3520.0 \pm 452.0

C: Control group,

E: Extrahepatic group

a: Significantly different from the control group at $P < 0.01$

reference: Hobara *et al.*, 1987a

- ***intravenous dogs***

The biliary excretion of TCA was studied in dogs. Groups of 5 mongrel dogs (male and female) were administered 25 mg/kg TCA via the right femoral vein. A control group received no treatment. The dogs' abdominal wall was cut and the choledoch duct was isolated for the collection of bile samples. Bile samples were collected every 30 minutes for up to 2 hours after TCA was administered. The amounts of excreted TCA and its metabolites (chloral hydrate, free-trichloroethanol, and conjugated trichloroethanol) were measured in each sample.

After 2 hours, a total of 1.32 ± 0.07 ml/kg of bile was excreted by the animals treated with TCA compared to 0.98 ± 0.06 ml/kg of bile excreted by the untreated controls. None of the bile samples showed any significant increases in the level of TCA or its metabolites in comparison to the controls. Approximately $1.0 \pm 0.07\%$ of the administered dose of TCA was excreted in the bile after 2 hours. None of TCA's metabolites were excreted in the bile. The authors concluded that TCA is slightly metabolized in the liver, and only small amounts of TCA are excreted in the bile [Hobara *et al.*, 1986a].

- **catheter insertion, dogs:**

In a study examining the absorption of trichloroethylene and its metabolites (including TCA), five adult mongrel dogs (male and female) were injected, via a catheter inserted into their bladder, with 61 mmol/L TCA in 20 ml of solution. A control group was injected with a buffer solution only. Blood samples were collected immediately before and 30, 60, 90, and 120 minutes after administration of TCA. Bile samples were obtained, as described in the previous bullet, every 30 minutes for two hours after dosing. In addition, the urinary bladder was bypassed with catheters inserted into the ureters, and urine samples were taken every 30 minutes for two hours after administration of TCA. After two hours, the remaining solution in the urinary bladder was drained through the catheter, and the volume was estimated to determine fractional water. The bladder was repeatedly washed with 1% phosphate buffer through the catheter, and this solution was also measured. The percentages of TCA and its metabolites were measured in the blood, bile, and urine samples, and in the remaining solutions taken from the bladder.

At the conclusion of the study, it was observed that TCA was metabolized to free trichloroethanol (F-TCE) and conjugated trichloroethanol (Conj-TCE). The blood and bile samples indicated that the concentration of TCA and Conj-TCE increased with time following TCA administration. There was no detectable concentrations of F-TCE in either the serum or the bile samples. The concentration of all three of these compounds (TCA, F-TCE, and Conj-TCE) was also found to increase over time in urine following dosing. Table 2 summarizes the increases in concentration of these compounds over time.

The percentage of TCA and other metabolites absorbed and excreted from the urinary bladder after 2 hours was determined. Two hours after administration of TCA, the solution remaining in the urinary bladder contained 99.68% TCA, 0.03% F-TCE, and 0.29% Conj-TCE. After 2 hours, it was determined that $65.5 \pm 6.4\%$ of the administered dose of TCA had been absorbed from the urinary bladder and $12.9 \pm 4.1\%$ of the water was absorbed from the urinary bladder. The percentage of water absorbed by the buffered control was $15.7 \pm 6.2\%$. The combined urinary and biliary excretion volume for TCA and its metabolites measured 2 hours after administration of TCA was $4.0 \pm 0.3 \mu\text{mol}$; the combined excretion rate was 0.5% (0.24% for urinary re-excretion). Two metabolites associated with trichloroethylene, F-TCE and Conj-TCE, were found in addition to TCA, although it is not clear where these compounds are produced since the dogs circulation was not impaired [Hobara *et al.*, 1988a; Hobara *et al.*, 1986b].

Table 2. The Concentration of TCA and Its Metabolites in Serum, Urine, and Bile Following Injection (by Catheter Insertion) in Dogs

Concentration of TCA and its Metabolites ($\mu\text{mol/L}$) at the Indicated Time After Administration (Minutes) of TCA

Sample	TCA				
	Metabolite	30	60	90	120
Serum	TCA	275.0 \pm 31.0	358.0 \pm 37.0	381.0 \pm 42.0	406.0 \pm 43.0
	F-TCE	*	*	*	*
	Conj-TCE	1.0 \pm 0.1	1.5 \pm 0.1	1.0 \pm 0.1	1.0 \pm 0.1
Urine	TCA	42.6 \pm 4.7	65.9 \pm 7.2	92.2 \pm 10.4	109.5 \pm 12.7
	F-TCE	2.1 \pm 0.3	3.2 \pm 0.3	3.9 \pm 0.4	4.3 \pm 0.4
	Conj-TCE	8.9 \pm 1.1	14.7 \pm 1.6	17.3 \pm 2.1	18.9 \pm 2.0
Bile	TCA	37.8 \pm 4.2	93.6 \pm 10.3	129.4 \pm 15.4	136.8 \pm 14.4
	F-TCE	*	*	*	*
	Conj-TCE	3.1 \pm 4.2	3.0 \pm 0.3	3.9 \pm 0.4	5.2 \pm 0.6

*The amounts present were below the limit of quantitation.

Reference: Hobara *et al.*, 1988a

- **catheter insertion, dogs:**

The absorption of TCA from the gallbladder was determined in an investigation of cholecystohepatic circulation using groups of 5 adult mongrel dogs (male and female). The test animals' gall bladders were washed with 1% phosphate buffer prior to injection, via catheter, with 10 ml of 1% (w/v) TCA in a buffered solution. A control group receiving the same treatment was given phosphate buffer, only. Arterial blood and urine samples were collected 30 minutes prior to administration of the test materials, and at 30-minute intervals for 2 hours after the test compound was given. The bile duct was isolated from the surrounding connective tissues and a catheter was inserted for collection of bile. Bile samples were collected every 30 minutes for 2 hours after the test material was given. Any bile remaining in the gallbladder after 2 hours was extracted, and its volume was determined. The percentage of TCA and other metabolites were measured in all samples and in the bile remaining in the gallbladder.

TCA was found in the serum, urine, and bile samples. Table 3 summarizes the observed levels of TCA in each of the samples collected. The concentration of TCA was observed to increase over time in serum and bile samples while the concentration of TCA in urine samples abruptly increased. About 40-50% of the administered TCA was absorbed from the gallbladder. The absorption rate of the cystic water was 25-30%. The percent absorption and the absorption rates were

calculated from the differences between the values of administered solutions and those of 2 hours after the administration. The authors state that there were no significant differences between the TCA and control groups.

After 2 hours, the remaining bile was collected and analyzed to determine the percentage of TCA and other metabolites. It was determined that the remaining bile contained 99.13% TCA, 0.35% free-trichloroethanol, and 0.17% conjugated-trichloroethanol. The combined urinary and biliary excretion ratio of TCA to the total amount remaining in the gallbladder was about 0.6%. (These calculations were done assuming each dog weighed 10 kg, and the volume of TCA excreted in bile and urine was 1 ml/kg/hour.)

The authors state that TCA is not efficiently absorbed from the gallbladder as only 40-50% was absorbed. Cystic water was absorbed (25-30%) indicating that its absorption is independent of substances dissolved in it. Metabolites of TCA were detected in the bile after 2 hours, but the location of TCA metabolism was not determined because circulation from the gallbladder to surrounding tissues was not disturbed. It was concluded that the gallbladder is capable of TCA absorption [Hobara *et al.*, 1987b].

Table 3: The Concentrations of TCA in the Serum or Blood, Urine, and Bile Following Injection (by Catheter Insertion) in Dogs

<u>Sample</u>	<u>Time After Administration (min. $\mu\text{mol/ml}$)</u>			
	<u>30</u>	<u>60</u>	<u>90</u>	<u>120</u>
Serum	38.4 \pm 4.3	71.1 \pm 5.8	93.6 \pm 6.2	107.5 \pm 7.5
Urine	27.5 \pm 4.1	45.3 \pm 4.6	58.6 \pm 5.5	73.2 \pm 6.4
Bile	4.2 \pm 0.6	23.5 \pm 2.1	32.8 \pm 2.3	44.1 \pm 4.7

Reference: Hobara *et al.*, 1987b

intestinal circulation system, dogs:

The absorption of TCA from the gastrointestinal tract was determined in dogs. Groups of 5 laparotomized, mongrel dogs (male and female) were administered a 500 ml dose of 16 mmol TCA/L TCA in 25% phosphate buffer into one of three parts of an intestinal circulation system using the jejunal, ileal, and colonic loops. Control groups were administered buffer only. Blood, urine, and bile samples were collected from the test animals every 30 minutes for 2 hours after administration of the test materials. Blood and urine samples were also collected 30 minutes prior to administration of TCA. Samples of the circulating solution were collected from an outlet in the system every 30 minutes until 2 hours after administration. The amount of TCA and its metabolites, free trichloroethanol (F-TCE) and conjugated TCE (Conj-TCE), were measured in each sample. The following biological and physiological parameters were evaluated: heart function (blood pressure and heart rate), blood chemistries (GOT < GPT < A1-P, erythrocyte and leukocyte counts, haematocrit, BUN, and creatinine). None of the biological or physiological parameters measured were altered by the surgical procedure.

The concentration of TCA and its metabolites measured in the blood and urine of the test animals is summarized in Table 4. Following administration of TCA into the jejunum, ileum, or colon, the concentration of TCA in the serum, urine, and bile increased over time. Levels of Conj-TCE remained constant in the serum, but increased over time in the urine and bile. Conj-TCE levels were lower than TCA levels. F-TCE was not detectable in serum or bile samples, but was detectable at low concentrations in the urine after 90 minutes (jejunum) and 120 minutes (ileum). The levels of TCA in the buffer decreased gradually after administration of TCA into the jejunum, ileum, or colon. The levels of the metabolites (Conj-TCE and F-TCE), however, increased in the three experiments. In the serum, urine, and bile samples, the concentrations of each substance (TCA, Conj-TCE, or F-TCE) were highest after administration of TCA into the jejunum, and lowest following administration of TCA into the colon. In the buffer, the concentrations of administered substance (TCA) were lowest after injection of TCA into the jejunum, and highest after administration of TCA into the colon. In the ileal and jejunal experiments, the concentration of F-TCE in the buffer was higher than that of Conj-TCE, while the opposite effect was observed in the colonic experiment.

Two hours after administration of TCA, the proportion of water absorbed by the jejunum, ileum, or colon was about 10%, and there were no significant differences between control and treatment groups in any region. The absorption of TCA was about 40-50% in the jejunum, and 30-40% in the ileum and colon. The amount of urine and bile excreted was constant (about 1 ml/kg/hour), and the combined biliary and urinary excretion ratios of TCA and its metabolites to the total amounts absorbed from the intestine were about 0.1-0.2% two hours after TCA administration. This value did not differ significantly among the three parts of the intestine. The authors of this study state that after absorption, TCA may be distributed and metabolized to other compounds which were not determined in

this study (CO₂). In support of this, the authors point to the low levels of TCA and its metabolites in the urine and bile, and to the low levels of TCA in the serum compared to the amounts of TCA absorbed [Hobara *et al.*, 1988b; Hobara *et al.*, 1988c].

Table 4. The Concentration of TCA and its Metabolites in the Serum and Urine After Administration of TCA in the Jejunum, Ileum, and Colon

Concentration of TCA and Metabolites (µmL/L) at the indicated time after TCA Administration (min)						
<u>Location</u>	<u>Sample</u>	<u>Metabolite</u>	<u>30</u>	<u>60</u>	<u>90</u>	<u>120</u>
Jejunum	Serum	TCA	102.0±11.4	134.0±13.6	148.0±15.1	179.0±18.4
		F-TCE	*	*	*	*
		Conj-TCE	2.3±0.3	3.1±0.3	3.5±0.4	3.3±0.3
	Urine	TCA	68.5±8.6	167.0±19.1	329.0±34.4	516.0±48.2
		F-TCE	*	*	2.6±0.3	3.9±0.5
		Conj-TCE	17.4±2.3	24.5±3.1	24.8±2.7	31.1±3.9
Ileum	Serum	TCA	64.1±8.4	96.8±10.7	119.0±12.3	140.0±15.9
		F-TCE	*	*	*	*
		Conj-TCE	*	*	1.2±0.1	2.2±0
	Urine	TCA	53.8±5.6	133.0±14.1	237.0±24.5	413.0±49.2
		F-TCE	*	*	*	1.7±0.2
		Conj-TCE	*	4.9±0.5	9.6±1.2	20.3±2.4
Colon	Serum	TCA	53.4±6.2	77.9±7.2	93.6±10.2	119.0±12.4
		F-TCE	*	*	*	*
		Conj-TCE	*	*	*	1.5±0.1
	Urine	TCA	44.6±5.4	105.0±12.2	174.0±20.6	326.0±34.4
		F-TCE	*	*	*	*
		Conj-TCE	*	*	4.2±0.5	10.5±0.9

* - The amounts present were below the unit of quantitation.

reference: Hobara *et al.*, 1988b

B. Acute

1. Human Data

No Data were found on the acute effects of either DCA or TCA in humans.

2. Animal Data

a) Dichloroacetic Acid

Acute LD₅₀ and skin and eye irritation data for DCA are described below in Table 5.

- ***oral, rats***

The acute toxicity of DCA was determined using rats. Groups of 4-6 male and female Sprague-Dawley rats were gavaged with three single 2 ml/kg doses of 0, 0.92, or 2.45 $\mu\text{mol/kg}$ DCA within a 24-hour period. The animals were sacrificed three hours after receiving their final dose and blood, liver, and kidney samples were collected and the concentrations of lactate and glucose levels were measured in each sample.

Changes in plasma glucose and liver lactate concentrations in all of the test animals were observed, but these changes were not significantly different from controls ($P < 0.05$). No changes were reported in kidney lactate concentrations in the animals. DCA, at both concentrations administered, significantly ($P < 0.05$) decreased the concentration of plasma lactate in both males and females. The author stated that female rats appeared to be more susceptible to the effects DCA than male rats. DCA-induced effects were compared to those induced by TCA in a parallel experiment (see Davis, 1990, below)[Davis, 1990].

Table 5: Acute LD₅₀ and Irritation Data for Dichloroacetic Acid in Animals

<u>Route Exposure</u>	<u>Species/Strain</u>	<u>No. of Animals/ Dose Group</u>	<u>Dose/Range (effect)</u>	<u>Reference</u>
Oral	Rat/NS	10*	4.48 g/kg (4.29-4.69 g/kg) (LD ₅₀)	Woodard <i>et al.</i> , 1941
Oral	Rat/NS	NS	2.82 g/kg (LD ₅₀)	Smyth <i>et al.</i> , 1951
Oral	Mice/Albino	10	5.52 g/kg (3.81-8.00 g/kg) (LD ₅₀)	Woodard <i>et al.</i> , 1941
Dermal	Rabbit/NS	NS	0.510 g/kg (LD ₅₀)	Smyth <i>et al.</i> , 1951
Dermal	Rabbit/NS	NS	10 mg/24 hr. (Moderate)	Smyth <i>et al.</i> , 1951
Ocular	Rabbit/NS	NS	50 µg (Severe)	Smyth <i>et al.</i> , 1951

*NS = Not specified

b) Trichloroacetic Acid

Acute LD₅₀ and irritation data for TCA are described below in Table 6.

• oral, rats:

The acute toxicity of TCA was determined in rats and compared to the toxicity of DCA (results of a parallel experiment, using DCA, are described above). An unspecified number of male and female Sprague-Dawley rats were gavaged with three single doses of 0, 0.92, or 2.45 µmol/kg TCA within a 24-hour period. The test animals were sacrificed 3 hours after the final dose was administered. Blood, liver, and kidney samples were collected to measure lactate and glucose levels.

In female rats, TCA significantly (P<0.05) decreased the level of plasma glucose in the high dose group (2.45 µmol/kg) and the liver lactate concentration in both treatment groups (0.92 µmol/kg and 2.45 µmol/kg). A significant (P<0.05) decrease in plasma lactate concentration was reported in both the males and females in the high dose group, and in females in the low dose group. No changes were reported in kidney lactate concentrations in the animals. The author stated that female rats appear to be more susceptible to the effects TCA than male rats. The effects of TCA on intermediary metabolism were compared to those of DCA and found to be similar (i.e., like DCA, TCA may activate pyruvate dehydrogenase or be metabolized to oxalate and glyoxalate) [Davis, 1990].

- **oral rats:**

In a 14-day study, the acute effects of TCA in drinking water (reagent grade) were studied in male and female rats (unspecified strain) exposed to TCA at concentrations of 0.04, 0.16, 0.63, or 2.38 g/L for 14 days. Control animals received water only. The food and water consumption of each animal was recorded daily. Urine samples were collected, and volume, osmolality, and solute excretion were measured. At the conclusion of the study, the animals were sacrificed, body weights recorded, and blood, kidney, and liver samples were collected to determine glucose and lactate concentrations.

TCA had significant effects on body weight in both males and females. A decrease in weight followed by an increase (day 7) parallel to the controls was observed in the high dose groups. For instance, in the first two days, females lost weight (from 246.0±5.0 g to 231.0±6.0 g), while the controls gained weight (from 237.0±4.0 g to 239.0±5.0 g). Decreased food and water consumption were also reported and were determined to be consistent with weight loss.

On day 7 of the study, urine osmolality increased and urine volume decreased in males from each treatment group. The high dose group excreted smaller amounts of more concentrated urine, which was consistent with the decreased water intake. The females exposed to TCA experienced a dose-related decrease in urine volume and, to a lesser degree, an increase of osmolality, such that solute excretion decreased. On day 7, the urine osmolality in high dose females was reported as being significantly ($P<0.05$) higher than the osmolality in the control group. The authors concluded that TCA impairs the ability to concentrate urine sufficiently to match urine output to decreased fluid intake. There were no significant changes in either liver or kidney lactate content or plasma glucose concentration from treatment with TCA.

The author stated that while intermediary metabolism was not affected by exposure to TCA, some sex-dependent effects on renal function were noted; renal function was maintained in male rats, but not in females. In females, urine was not as concentrated as it should have been based on the reduced volume observed, and total excretion of osmoles decreased, suggesting interference with electrolyte homeostatic mechanisms. The author concluded that there may be a sex difference in the handling of TCA or in the responsiveness to some of its effects. Although the reasons for these differences have not been resolved, different rates of metabolism has been suggested as a possible cause [Davis, 1990].

- **dermal, rats:**

A study was performed on rats (strain and sex unreported) to determine the cardiotoxicity of TCA. Seven rats were administered a dose of 50% TCA to an epilated area of the abdomen proportional to the average human face (approximately 5% of the total body surface area). The animals' cardiac rhythm, rate, and arterial pressure were monitored. Once those parameters had stabilized (a variable period up to 1 hour), the entire abdomen was covered with the test compound, and cardiac responses were again recorded.

After the initial exposure to TCA, none of the animals were observed to have any significant cardiac arrhythmias or changes in heart rate. Two animals exhibited a moderate drop in blood pressure which increased prior to continuation of the study. When the test compound was applied to the entire abdomen, the blood pressure of 5/7 animals was observed to decrease within 10 minutes, with a mean decline of 39 mm Hg (the average initial systolic pressure was 116 mm Hg). There were also some rate changes observed but these were inconsistent with the changes observed in blood pressure. Two of the test animals were observed to have cardiac rhythm changes and died as a result of these changes. In one of those animals, the fatality was due to exsanguination (uncontrolled surgical bleeding); the other animal awoke during application, was reanesthetized, and died 14 minutes after application of TCA to the entire abdomen. The second death was most likely due to an idiosyncratic reaction to stress, fear or pain, or anesthetic overdose. The authors concluded that TCA resulted in the depression of blood pressure especially when applied to a large area, but was not cardiotoxic to rats [Stagnone *et al.*, 1987].

- **dermal, mice:**

In an experiment examining the depigmenting effect of laundry ink on the hair of CBA mice, TCA (1/1000 in an unspecified solvent) was applied to the back of the heads and necks of 5 female CBA/J agouti mice. All 5 of the animals exhibited local irritation and hair loss. Within 5 days of the application of TCA, all 5 animals died [Shelley and Raque, 1972].

- dermal mice:

TCA was used to induce epidermal activity in an investigation to identify the site(s) of action of anthralin, an antipsoriatic drug. Forty hairless mice (male and female) were painted on half of their backs with 25% TCA in an unspecified solvent (to induce epidermal activity), the other half of their backs was untreated and served as a control. Eight mice/group received intraperitoneal injections of tritiated thymidine (25 μ Ci/mouse) at 0, 4, 24, 48, and 72 hours after the TCA was applied, and were sacrificed 2 hours later. Another 40 mice were painted with 0.4% anthralin in Lassar's paste over half their backs and with both TCA and anthralin on the other half. These mice were also injected with irradiated thymidine in the same manner as the first group. Then, a section of skin (4.5 cm²) was taken from each site, the epidermis was removed, and the radioactivity present in the epidermis was counted.

A significant increase ($P < 0.05$) in the uptake of tritiated thymidine (cpm/mg) was seen during the first period (0-2 hours) in the epidermis treated with TCA (140.49 ± 28.06) compared to the controls (84.62 ± 12.17). All subsequent test periods showed baseline increases. With anthralin, there was no increase in the uptake of thymidine at either 0-2 hours or 4-6 hours; but, uptake was doubled during the 24-26 hour period with TCA and anthralin (48.26 ± 6.96) compared to anthralin alone (28.82 ± 4.30 , $P < 0.025$) and at the 48-50 hour period (29.59 ± 5.07 (anthralin); 49.60 ± 14.83 (TCA + anthralin), $P < 0.03$). The authors noted that stimulation of epidermal cellular proliferation by TCA, particularly the S phase, occurs within 2 hours [Heng and Beck, 1982].

- dermal mice:

In a 1935 study done by Bereblum, no incidence of tumor formation, irritation, or death was reported in mice (unspecified strain and sex) administered an unspecified concentration of TCA in acetone, 3 times weekly for one month. No other data were reported [Hartwell, 1951].

Table 6. Acute LD₅₀/Irritation Data for Trichloroacetic Acid in Animals

<u>Route Exposure</u>	<u>No. of Animals Species/Strain</u>	<u>Dose/Range Per Dose Group</u>	<u>(effect)</u>	<u>Reference</u>
Oral	Rat/NS	10	3.32 g/kg (3.16-3.48 g/kg) (LD ₅₀)	Woodard <i>et al</i> , 1941
Oral	Rat/NS	NS	3200-5000 mg/kg (LD ₅₀)	Worthing, 1987
Oral	Rat/NS	NS	5060 mg/kg (LD ₅₀)	Meister, 1990
Oral	Rat/NS	NS	5000 mg/kg (LD ₅₀)	Meister, 1990
Oral	Mice/Albino	10	4.97 g/kg (4.70-5.26 g/kg) (LD ₅₀)	Woodard <i>et al</i> , 1941
Oral	Mice/NS	NS	5640 mg/kg (LD ₅₀)	Worthing, 1987
Intraper.	Mice/NS	NS	500 mg/kg (LD ₅₀)	Off. Fed. Reg., 1989
Subcut.	Mice/NS	NS	270 mg/kg (LD ₅₀)	RTECS, 1991
Dermal	Rabbit/NS	NS	210 µg (mild)	RTECS, 1991
Ocular	Rabbit/NS	NS	3500 µg (severe)	RTECS, 1991

NS: not specified

C. Prechronic

1. Human Data

No data were found in the literature on the prechronic effects of either DCA or TCA in humans.

2. Animal Data

a) Dichloroacetic Acid

- oral, rats:

The toxic effects of DCA were studied in rats following subchronic exposure. Male Sprague-Dawley rats were divided into four treatment groups (10 animals/group) and were given DCA in their drinking water at concentrations of 0, 50.0, 500.0, or 5000.0 ppm for 90 days. The test animals were observed daily throughout the duration of the study. Their body weights were recorded weekly and water consumption was measured after approximately 60 days of exposure.

At the conclusion of the study, the test animals were sacrificed and studies were performed to assess the toxic effects from DCA exposure. Serum samples were collected and the serum biochemical profile (blood urea nitrogen (BUN), creatinine, glucose, alanine-amino transferase (SGPT), alkaline phosphatase, cholesterol, total protein, albumin, calcium, phosphorus, creatinine phosphokinase (CPK), and gamma glutamyl transpeptidase (GGT)) was assessed. Immunological parameters including T cell-dependent IgG antibody production, delayed-type hypersensitivity, natural killer cell cytotoxicity, and production of macrophage-derived prostaglandin E₂ (PGE₂) and lymphocyte-derived interleukin 2 (IL2) were examined. In addition, necropsies were performed on all the animals, and the individual organ (liver, kidney, spleen, thymus, and testes) to body weight ratios were determined. Samples of brain, heart, lung, pancreas, adrenals, lymph nodes, gastrointestinal tract, urinary bladder, muscle, skin, liver, kidney, spleen, thymus, and testes were collected and stained for histological examination. Liver samples were collected to measure the peroxisomal *B*-oxidation activity.

The body weights for the animals receiving 500.0 and 5000.0 ppm DCA were significantly ($P \leq 0.05$) reduced in a dose-dependent manner compared to the controls. Significant changes were evident after 4 weeks (5000.0 ppm) and 9 weeks (500.0 ppm). Significant ($P \leq 0.05$) increases in organ/body weight ratios were observed in the liver and kidney (500.0 and 5000.0 ppm) and spleen (5000.0 ppm). There were no treatment-related changes observed in either the thymus or testes. A decrease in water consumption was observed after 2 months of exposure in all test groups; however, this decrease was significant ($P \leq 0.05$) in the 500.0 and 5000.0 ppm treatment groups, only.

The total serum protein levels were significantly ($P \leq 0.05$) depressed in all of the animals treated with DCA in comparison to the controls. The measured total serum protein in the controls was 7.2 ± 0.1 g/dl, and in the DCA treatment groups, the following serum protein levels (in g/dl) were measured: 6.8 ± 0.1 (50 ppm), 6.7 ± 0.1 (500.0 ppm), and 6.6 ± 0.1 (5000.0 ppm). Significant increases ($P \leq 0.05$) in alkaline phosphatase levels (215.5 ± 17.6 IU/l (500.0 ppm)) and 293.7 ± 17.6 IU/l (5000.0 ppm) and SGPT levels (82.4 ± 9.9 IU/l (5000.0)) were observed compared to controls (alkaline phosphatase- 183.7 ± 21.1 IU/l and SGPT- 56.5 ± 11.9 IU/l). Peroxisomal *B*-oxidation activity in the liver was observed to be significantly ($P \leq 0.05$) increased in the 5000.0 ppm dose group only. There was no evidence of treatment-related changes in any of the immunological parameters studied.

Necropsy of the animals revealed that the liver weights of the animals given 5000.0 ppm DCA (21.4 ± 0.7 g) were significantly ($P \leq 0.05$) elevated compared to the controls (15.7 ± 0.9 g), and liver enlargement was grossly evident. Microscopic evaluation revealed focal areas of hepatocellular enlargement. This swelling was most severe in the animals receiving 5000.0 ppm DCA. Other treatment-related abnormalities observed in the high dose group included pockets of proteinaceous fluid in the liver, diffuse degeneration of tubular epithelium and cells of the glomeruli in the kidney, and enlarged spleens.

The authors suggest that the results of this study indicate that long-term exposure to DCA produces substantial systemic toxicity to the liver and kidney. According to the authors, the amount of DCA likely to cause harm exceeds levels of DCA that are expected to be found in the environment and chlorinated drinking water. Mather *et al.*, concluded that the data indicate that the kidney and liver are the major target organs for DCA [Mather *et al.*, 1990].

b) Trichloroacetic Acid

• oral, rats:

The effects from prechronic exposure to low concentrations of TCA were studied in rats. Four groups (10 animals/group) of male Sprague-Dawley rats were given TCA, daily, in their drinking water at concentrations of 0, 50.0, 500.0, or 5000.0 ppm for 90 consecutive days. A serum biochemical profile was done on all of the animals surviving at the end of the 90 day study. Tests included blood urea nitrogen, creatinine, calcium, phosphorus, total protein, albumin, glucose, cholesterol, alkaline phosphatase, creatinine phosphokinase, and alanine aminotransferase. At the conclusion of the study, the animals were sacrificed, necropsied, and examined histopathologically. The animals' weights were recorded prior to treatment and at the time of sacrifice. Weights obtained from the liver, kidneys, spleen, and thymus at necropsy were used to determine organ to body weight ratios. All gross lesions and tumors were recorded. The brain, heart, lung, pancreas, adrenals, lymph nodes, gastrointestinal tract, urinary bladder, muscle, skin, kidney, spleen, thymus, and testes were fixed, processed, and stained with hematoxylin and eosin to evaluate the number of GGT-positive foci.

There were no significant differences in body or organ weights as a percentage of body weights between the test groups and the control group. In addition, there were no gross lesions that were attributed to the TCA exposure. Microscopic examination of the tissue was unremarkable. The serum cholesterol levels were significantly ($P \leq 0.05$) decreased in all of the TCA treatment groups compared to the controls. A significant ($P \leq 0.05$) increase, of approximately 50% over controls, was seen in serum alanine aminotransferase levels in the animals dosed with 5000.0 ppm TCA. No other significant differences between experimental groups were noted. The author stated that the general toxicity of TCA, as determined by the parameters evaluated, is reasonably low in rats. The procedures and results for other studies which were conducted in conjunction with this 90-day study are described in sections V.D.2 and V.G.3 [Parnell, 1986].

- **oral rats:**

The toxic effects of TCA in rats were studied in a 90-day investigation in conjunction with the 90-day study on DCA (Mather *et al.*, 1990) described previously. Male Sprague-Dawley rats were divided into four groups of 10 animals each and given doses of TCA in their drinking water at concentrations of 0, 50.0, 500.0, or 5000.0 ppm. The test animals were observed daily throughout the duration of the study. Their body weights were recorded weekly and the amount of water consumption was measured after approximately 60 days of exposure.

At the conclusion of the study, the test animals were sacrificed and studies were performed to assess the toxic effects from TCA exposure. Serum samples were collected and the serum biochemical profile (blood urea nitrogen, creatinine, glucose, alanine-amino transferase (SGPT), alkaline phosphatase, cholesterol, total protein, albumin, calcium, phosphorus, creatinine phosphokinase, and gamma glutamyl transpeptidase) was assessed. Immunological parameters including T cell-dependent IgG antibody production, delayed-type hypersensitivity, natural killer cell cytotoxicity, and production of macrophage-derived prostaglandin E₂ (PGE₂) and lymphocyte-derived interleukin 2 (IL2) were evaluated. Necropsies were performed and individual organ (liver, kidney, spleen, thymus, and testes) to body weight ratios were determined. Samples of brain, heart, lung, pancreas, adrenals, lymph nodes, gastrointestinal tract, urinary bladder, muscle, skin, liver, kidney, spleen, thymus, and testes were collected and stained for histological examination. Liver samples were collected to measure the peroxisomal B-oxidation activity.

There were no significant changes in body weight for any of the test animals. A significant ($P \leq 0.05$) decrease in water consumption was observed at the 2 highest levels. Significant ($P \leq 0.05$) increases in organ/body weight ratios were observed in the liver ($4.35 \pm 1.10\%$) and kidney ($0.41 \pm 0.01\%$) of the 5000.0 ppm group compared to the control group (liver ($4.07 \pm 0.10\%$), and kidney ($0.37 \pm 0.01\%$)). There were no treatment-related weight changes observed in either the spleen,

thymus, or testes. No consistent differences were seen in serum chemistry parameters of TCA-treated animals. The livers of animals exposed to 5000.0 ppm TCA had significantly ($P \leq 0.05$) elevated peroxisomal *B*-oxidation activity. There was no evidence of treatment-related changes in the immunological parameters evaluated.

Microscopic evaluation revealed focal areas of hepatocellular enlargement among the high dose group. The intracellular swelling was less severe than that observed in the parallel study conducted by Mather *et al.* (1990) on DCA (described above). It was observed that the livers of the TCA-treated rats accumulated less glycogen. The authors suggest that the results of this study indicate that long-term exposure to TCA produces substantial systemic toxicity to the liver and kidney. Mather *et al.*, report that the amount of TCA likely to cause harm exceeds levels that are expected to be found in the environment and chlorinated drinking water [Mather *et al.*, 1990].

- **dermal rats:**

The effects of TCA when applied dermally to oral mucosa, submucosal connective tissue, pulp, alveolar bone, and periodontium were assessed in rat. The second objective of this study was to provide a theoretical basis to an apparently satisfactory clinical adjunctive measure in the treatment of invasive cervical resorption. Twenty-eight male Sprague-Dawley Porten strain rats were topically treated with TCA (90% in aqueous solution) for 30 seconds at two locations: 1) midline junction of the soft and hard palate and 2) a cavity prepared between the first and second molar, originating at the palato-gingival margin and continuing 3 mm into underlying periodontal ligament, bone, and tooth. A control cavity was prepared on the contralateral side and irrigated with saline. A group of untreated rats served as controls. The animals were sacrificed at 5 and 30 minutes, 1 and 16 hours, and 1, 2, 5, 7, 10, 14, 21, 28, and 42 days. Macroscopic observations were recorded at each experimental period. The animals were decapitated, the mandible and associated soft tissue were removed, and the cranium and soft tissue were fixed for histological evaluation.

No signs of toxicity in the test animals were apparent at any time during the study. The formation of white lesions on the soft palate was observed within a few seconds after TCA was applied. Histological examinations of the soft palate revealed coagulation necrosis with a distinct line of demarcation between affected and nonaffected tissues. Separation of the necrotic tissue from the rapidly regenerating tissue occurred on, or shortly after, the second day. The healing process continued uninterrupted until there was no evidence of residual pathology (on approximately the fifth day). The authors suggest that the lack of a chronic inflammatory response in the tissues beneath the well-defined area of coagulation necrosis was a contributing factor towards the rapid and uncomplicated repair.

Minor differences in the healing of gingival tissue, periodontium, subepithelial tissues, alveolar bone, and pulp between the test and control cavities were

observed early in the study. The initial reaction to TCA (particularly 30 minutes and 1 and 16 hours) of the gingival and palatal mucosa were similar to the reactions observed in the soft palate. Red blood cells were absent in the test cavity after 5 minutes in one of the rats, but the authors repute that this may have been due to an artifact or a result of the hemostyptic effect of TCA. At all other experimental periods, the tissue responses in both the experimental and control sites followed a predictable pattern of repair. There were no observable reactions to TCA in the tooth pulp or the root.

The authors state that the results of this study indicate that there are no substantial differences between the responses of rat tissue to mechanical trauma and a 30 second application of TCA and controls receiving the same trauma. They further suggest that clinical application of TCA in the treatment of invasive cervical resorption will apparently not be hampered or undermined by any residual, medicament-related delayed healing of the traumatized periodontal and related tissues [Heithersay and Wilson, 1988].

- ***dermal mice:***

TCA was one of 27 compounds used in a preliminary validation study of the final design of the mouse ear sensitization assay. Sixteen female Balb/c mice received a 10% dose of TCA in ethanol on both sides of their right ear on days 0 and 2. On day 2 they also received a scapular subcutaneous injection of 0.5 ml Freund's adjuvant. On day 9, an induction dose of 10% TCA was administered topically to both sides of the left ear and the ear thickness was measured immediately before compound administration and after 24 hours. The measured ear thickness (in mm) immediately before the challenge was 21.81 ± 1.69 ; after 24 hours the thickness was determined to be 21.55 ± 1.84 . (Ear thickness was determined to be 21.66 ± 1.5 and 21.69 ± 1.91 immediately before and 24 hours after challenge in control animals administered ethanol only.) The authors state that these results are not significant and that TCA is a nonsensitizing compound in the mouse ear sensitization assay [Descotes, 1988].

D. Chronic/Carcinogenicity

1. Human Data

No data were found on the chronic effects/carcinogenicity of DCA or TCA in humans.

2. Animal Data

a) Dichloroacetic Acid

- **oral mice** :

The carcinogenicity of DCA was studied in male B6C3F1 mice. The mice were randomly separated into 4 groups of 50 mice each (3 test groups and 1 control group). The test animals received daily doses of 0.05, 0.5, or 5.0 g/L DCA in their drinking water and the control group received 2.0 g/L sodium chloride in their drinking water. For the first month, water intake and body weights were recorded weekly. After the first month, these data were recorded monthly.

After 4, 15, 30, and 45 weeks, 5 animals from each treatment group were sacrificed. After 60 weeks of treatment, 9 animals from the 0.05 g/L DCA, 0.5 g/L DCA, and control groups; and 30 animals from the 5.0 g/L DCA group were sacrificed. The remaining animals continued to receive treatment for 15 more weeks. At the conclusion of the study (week 75), these animals were sacrificed. Complete necropsies and histopathological examinations of all the test animals were performed. The weights and gross lesions (discolorations, surface irregularities, nodular changes, and masses) of the animals' liver, kidney, testes, and spleen were recorded. Lesions and surrounding tissues were excised and fixed for histopathological analysis.

The frequency at which hyperplastic nodules (HN), hepatocellular adenomas (HA) and hepatocellular carcinomas (HC) occurred were recorded both individually and summed as the total liver tumor prevalence. Tumor prevalence was calculated as the ratio of the number of animals with a neoplastic lesion to the number of animals examined.

The difference in the amount of water consumed by the control group and test animals receiving 0.05 and 0.5 g/L was minimal; however, a 60% decrease in water intake, compared to the control, was observed in the high dose group (5.0 g/L) group. The daily water intakes (ml/g body wt/day \pm SD) measured over the course of the study were 0.16 \pm 0.01 (control group), 0.15 \pm 0.01 (0.05 g/L DCA), 0.14 \pm 0.01 (0.5 g/L DCA), and 0.09 \pm 0.001 (5.0 g/L DCA). The mean daily dose of DCA was calculated over the testing period, for each test group, and determined to be 7.6 (0.05 g/L), 77 (0.5 g/L), and 486 (5.0 g/L) mg/kg/day. Many daily doses calculated at the beginning of the exposure period were high relative to those at the end of exposure.

There were no observable differences in weight gain between the control group and the animals treated with 0.05 g/L and 0.5 g/L DCA. The final body weights for the high dose group (5.0 g/L DCA) were significantly (17%, P<0.02) lower than the control group. There were no differences in the survival rates between the test groups.

The relative liver weights (RLW) at 60 weeks for the 5.0 g/L and 0.5 g/L groups were significantly ($P<0.02$) increased (351% and 118%, respectively). The large increase seen in the 5.0 g/L group was due, in part, to the presence of proliferating nodules and neoplastic lesions. The RLW of the 0.05 g/L DCA treatment group did not vary significantly from the control group values after 60 or 75 weeks. There were no changes seen in the relative weights of the kidney, spleen, or testes.

The three types of proliferative lesions observed in the liver were HN, HA, and HC. A significant ($P<0.0001$) dose-related trend was found for the relationship between age and the incidence of liver tumors. HA was first seen at 45 weeks in the 0.5 g/L dose group and HC was observed after week 60 in the 0.05 g/L DCA group. Tumors in the control group were only observed after 75 weeks. The number of proliferative lesions in each dose group, with exception of the 0.5 g/L DCA group ($P=0.19$), was significantly different from the control (0.05 g/L ($P<0.05$), and 5.0 g/L ($P<0.001$)). A significant ($P<0.001$) increase of HN was found in the high dose group at 60 weeks. The incidence of HN in this group was 83% (1.27 HN/animal). HN's were also observed in the low dose group, but the incidence was insignificant (0.02 HN/animal). HA and HC were first seen in the high dose group at 45 weeks. At 60 weeks, the prevalence of HA (80%, 2.30 HA/animal), HC (83%, 2.20 HC/animal), and total neoplasia (90%, 4.50 tumors/animal) was significantly ($P<0.001$) increased in comparison to the control. There were no significant increases in the total number of neoplasms between the 0.05 g/L DCA (24.1%, $P>0.07$), 0.5 g/L DCA (11.1%, $P<0.10$), and control group.

The authors state that the results of this study confirm the carcinogenicity of DCA in drinking water in mice following a 60-week exposure. They further stated that DCA exhibits a threshold response curve that, once crossed, a maximum tumor prevalence is rapidly attained [DeAngelo *et al.*, 1991].

- **oral mice:**

The carcinogenic effects of DCA were studied by DeAngelo *et al.* in an investigation run concurrently with the study described in the previous bullet. Groups of 50 male B6C3F1 mice were administered either 3.5 g/L DCA or 1.5 g/L acetic acid (control) in their drinking water. (See previous bullet for a description of the experimental design.) Five test animals from each group were sacrificed after 4, 15, 30, or 45 weeks. After 60 weeks, 12 mice in the DCA treatment group and 10 mice from the control group were sacrificed.

The daily water intake for the animals was measured over the course of the study. The animals treated with 3.5 g/L DCA consumed significantly less water than the acetic acid controls (0.11 ± 0.01 and 0.16 ± 0.02 ml/g body wt/day \pm SD, respectively). The calculated mean daily dose of DCA for the 3.5 g/L group was 410 mg/kg/day.

The final body weights for the animals treated with DCA were significantly ($P < 0.001$) lower than control weight. The relative liver and kidney weights of DCA-treated mice was significantly ($P < 0.02$) increased compared to the controls at 75 and 65 weeks, respectively. There were no significant changes in the testes or spleen weights.

The total number of liver tumors was observed to be increased relative to age in a dose-related trend. The number of tumors found in the 3.5 g/L DCA group was significantly ($P < 0.001$) increased over the control group. At 60 weeks, the 3.5 g/L mice were observed to have HN (58%, 0.92 HN/mouse), HA (100%, 2.33 HA/animal), and HC (67%, 1.67 HC/animal) for a total of 4.0 neoplasms/animal. The prevalence of tumors observed in this study did not vary significantly with the findings reported above for mice dosed with 5.0 g/L DCA. The authors report that the results obtained during this investigation confirmed the carcinogenicity of DCA in mice following administration in the drinking water for 60 weeks [DeAngelo *et al.*, 1991].

- **oral mice :**

The ability of DCA to either promote ethylnitrosourea (ENU)-initiated hepatocarcinogenesis or act as a complete carcinogen was studied in neonatal mice. Groups of 15-day old male B6C3F1 mice were initiated with a single, intraperitoneal injection of ENU at a concentration of 0 (N=70), 2.5 (N=98), or 10.0 $\mu\text{g/g}$ (N=23). When the test animals were 4 weeks old, they began receiving DCA in their drinking water at concentrations of 2.0 or 5.0 g/L for 61 weeks. The groups were divided as follows: 29 animals (2.0 $\mu\text{g/L}$ DCA/2.5 $\mu\text{g/g}$ ENU), 32 animals (5.0 g/L DCA/2.5 $\mu\text{g/g}$ ENU), or 26 animals (5.0 g/L DCA/0.0 $\mu\text{g/g}$ ENU). Thirty-eight animals serving as positive controls were given 0.5 g/L sodium phenobarbital (PB), a known tumor promotor, and 66 negative controls received 2 g/L sodium chloride (NaCl) in the drinking water. The control groups were divided into the following groups: the positive control group-22 animals (PB/0.0 $\mu\text{g/g}$ ENU) and 16 animals (PB/2.5 $\mu\text{g/g}$ ENU) and the negative controls-22 animals (NaCl/0.0 $\mu\text{g/g}$ ENU), 21 animals (NaCl/2.5 $\mu\text{g/g}$ ENU) and 23 animals (NaCl/10.0 $\mu\text{g/g}$ ENU).

At the conclusion of the study (animals 65 weeks old), the test animals were sacrificed. At necropsy, the body, liver, and kidney weights were recorded and the animals were examined for grossly visible lesions including: discolorations, surface irregularities, nodular changes, and/or tumor masses. All lesions were measured, dissected out, and fixed in formalin for histopathological evaluation; nontumorous sections of the liver were also fixed for evaluation of pathologic and neoplastic changes.

The results were expressed as the number of animals with adenomas and hepatocellular carcinomas (HCC) and the incidence of adenomas and HCCs per animal (see Table 7 which summarizes the incidences of adenomas and HCCs observed). The animals receiving 5.0 g/L DCA had significantly reduced body weights and significantly increased ($P < 0.001$) liver weights, irrespective of ENU-initiation. The 2.0 g/L DCA animals had liver weights which were significantly increased ($P < 0.001$), but their body weights were unaffected (irrespective of ENU). The increased liver weights in this group were associated with an increased incidence of tumors in DCA-treated animals. The kidney weights in the high-dose groups were significantly decreased ($P < 0.001$).

DCA significantly increased the incidence of animals with adenomas and HCCs and the number of adenomas and HCCs per animal at both DCA concentrations tested ($P < 0.001$ [Herren-Freund and Pereira, 1987]) compared to the negative control groups. There was a dose-related increase in adenomas, but the increased incidence of HCCs was not found to be dose-related. The increase in adenomas and HCC occurred in the presence and absence of ENU. The authors concluded that DCA is a complete hepatocarcinogen in mice [Herren-Freund and Pereira, 1987; Herren-Freund *et al.*, 1987].

Table 7: Incidence of Adenomas and Hepatocellular Carcinomas in Mice Treated with Dichloroacetic Acid

(ENU/Promotor) ($\mu\text{g/g}$) (g/l)	Animals with Adenomas (%)	No. of Adenomas Per Animal ^a	Animals with Carcinomas	No. of Carcinomas (%)Per Animal ^b
DCA (2.5/5)	31 (97%)*	5.34 \pm 0.52*	25 (72%)*	1.47 \pm 0.24*
DCA 2.5/2	22 (76%)*	1.41 \pm 0.22*	19 (66%)*	1.17 \pm 0.22*
DCA 0/5	25 (96%)*	4.58 \pm 0.51*	21 (81%)*	1.69 \pm 0.29*
NaCl 10.0/2	9 (39%)	0.52 \pm 0.15	9 (39%)	0.57 \pm 0.20
NaCl 2.5/2	1 (5%)	0.05 \pm 0.05	1(5%)	0.05 \pm 0.05
NaCl 0/2	2 (9%)	0.09 \pm 0.06	0 (0%)	0.00
PB 2.5/500	1 (6)	0.06 \pm 0.06	0 (0%)	0.00
PB 0/500	2 (9%)	0.09 \pm 0.06	0 (0%)	0.00

* indicates the results are significantly ($p < 0.001$) different from the appropriate NaCl control group.

a,b mean \pm standard deviation

reference: Herren-Freund *et al.*, 1987

b) Trichloroacetic Acid

- oral, rats :

The ability of TCA to initiate and/or promote liver carcinogenesis was determined in short term initiation-promotion assays using male Sprague-Dawley rats. Both the GGT (gamma glutamyl-transpeptitase)-positive foci initiation bioassay and the hepatic peroxisomal beta-oxidation assay were used to determine the carcinogenic potential of TCA. Gross necropsy and histopathologic evaluations were performed on the animals at the time of sacrifice following dosing (described below) to observe any changes relative to exposure to TCA . The experimental animals were divided into a total of 14 groups, the initiation groups and promotion groups were designated as A-G and M-S, respectively. All of the experimental animals were 2/3 partial hepatectomized, except groups F and Q which received sham operations (no hepatic tissue removed). Two groups of animals (group H and group T), which received no experimental manipulation, served as negative age-matched controls.

Initiation Protocol: Twenty-four hours after the surgical procedure a single 10.0 mg/kg dose of diethylnitrosamine (DEN) (via intragastric intestation) or 1500 mg/kg TCA (via gavage) were administered to group A and group B, respectively. The remaining groups received 5000.0 ppm TCA in their drinking water for 10 (group C), 20 (group D), or 30 (group E and F) days. Two weeks after the initiation period, all of the animals were given 500.0 ppm phenobarbital (PB) daily in their drinking water for the remainder of the study. The test animals were sacrificed randomly at specific intervals during the experiment: 24 hours after the end of initiation, 24 hours prior to the start of promotion, and at 3, 6, and 12 month intervals after beginning promotion.

Promotion Protocol: Twenty-four hours after being partially hepatectomized or sham operated, animals were administered either DEN at 10 mg/kg body weight by oral gavage or distilled water (groups Q and R, respectively). Two weeks later, the test animals (except group R) received either 500.0 ppm PB (group M) or TCA at concentrations of 50.0 (group N), 500.0 (group O), or 5000.0 (groups P and Q) ppm daily in their drinking water. Animals were randomly selected and sacrificed at week 2, or 1, 3, 6, or 12 months during the promotion period.

The negative control groups (group H and group T) were sacrificed at 12 months only, due to lack of animals in these groups. At the time of sacrifice, the animals were necropsied and evaluated histopathologically. Their weights prior to treatment and at time of sacrifice were recorded. The liver, kidneys, spleen, and thymus were weighed at necropsy and organ-to-body weight ratios were calculated. All gross lesions and tumors were recorded. The brain, heart, lungs, kidneys, spleen, thymus, pancreas, adrenals, testes, lymph nodes, gastrointestinal tract, urinary bladder, muscle, and skin were fixed in 10% buffered formalin, processed, stained, and examined under the light microscope.

To quantify enzyme-altered foci (EAF), liver samples were collected from each test group and stained for GGT activity. Foci positive for GGT containing 9 or more nuclei were counted. The tissue area and number of foci were reported. (A peroxisomal beta oxidation assay was also carried out as part of this investigation. The experimental protocol and results for this assay are described in section V.G.3.)

TCA did not significantly increase GGT-positive foci initiation in the initiation bioassay. Only in the positive control group A was there a significant increase in GGT-positive foci. The mean area of the GGT-positive foci for group A was consistently larger than those of other groups. The initiator control group (group G) had almost no GGT-positive foci compared with the negative age-matched controls. The four TCA treatment groups (B, C, D, and E) did not significantly induce GGT-positive foci.

In the promotion bioassay, the positive control group M was observed to have significantly increased GGT-positive foci. In contrast to group A (initiation positive control), group M (promotion) showed an increase in the number of GGT-positive EAF. The three TCA promoted groups (N, O, and P) showed a similar pattern. At the 3 month sampling interval, group N (low dose) was observed to have significantly ($P \leq 0.05$) higher foci induction than the negative control groups (Q, S, and R). At the 3-month sampling interval, group P (high dose) also exhibited a significantly ($P \leq 0.07$) increased level of foci induction compared to the negative control group R, however, the increases observed in group O were not statistically significant. At the 6 and 12 month intervals, foci induction was increased significantly ($P \leq 0.05$) in each of the TCA promotion groups. The mean GGT-positive foci areas increased in size at each sampling within all experimental groups. The largest mean areas, however, was observed in the positive control (group M).

Necropsy of the animals showed no significant differences in body or organ (spleen, liver, or kidney) weights among animals treated with TCA using either the initiation or promotion protocols at any of the sampling times. A significant ($P \leq 0.05$) increase in liver weights was detected in the positive control (group M) through the 6 month sampling period. No gross pathological lesions were attributed to TCA exposure. The author stated that the results indicate that TCA may possess only weak promoting activity in rat liver. The procedures and results for other studies which were conducted in conjunction with this carcinogenicity study are described in V.C.2 and V.G.3 [Parnell, 1986; Parnell *et al*, 1986; Parnell *et al*, 1988].

- **oral mice:**

The ability of TCA to either promote ethylnitrosourea (ENU)-initiated carcinogenesis or act as a complete carcinogen was studied in conjunction with a carcinogenicity investigation described previously for DCA (Herren-Freund *et al.*, 1987). Groups of 15-day-old B6C3F1 male mice were initiated with a single, intraperitoneal injection of ENU at a concentration of 0 (N=66), 2.5 (N=94), or 10.0 µg/g (N=51). When the test animals were 4 weeks old, they began receiving TCA in their drinking water at concentrations of 2.0 or 5.0 g/L for the next 61 weeks. The groups were divided as follows: 33 animals (2.0 g/L TCA/2.5 µg/g ENU), 23 animals (5.0 g/L TCA/2.5 µg/g ENU), 28 animals (5.0 g/L TCA/10.0 µg/g ENU), or 22 animals (5.0 g/L TCA/0.0 µg/g ENU). Thirty-eight animals serving as positive controls were given 0.5 g/L sodium phenobarbital (PB), a known tumor promotor, and 66 negative controls received 2.0 g/L sodium chloride (NaCl). The control groups were divided into the following groups: the positive control group-22 animals (PB/0.0 µg/g ENU) and 16 animals (PB/2.5 µg/g ENU) and the negative controls-22 animals (NaCl/0.0 µg/g ENU), 21 animals (NaCl/2.5 µg/g ENU), and 23 animals (NaCl/10.0 µg/g ENU).

At the conclusion of the study (animals 65 weeks old), the test animals were sacrificed. At necropsy, the body, liver, and kidney weights were recorded and the animals were examined for grossly visible lesions including discolorations, surface irregularities, nodular changes, and/or tumor masses. All lesions were measured, dissected out, and fixed in formalin for histopathological evaluation; nontumorous sections of the liver was also fixed for evaluation of pathologic and neoplastic changes. The results were expressed as the number of animals with adenomas and hepatocellular carcinomas (HCC) and the incidence of adenomas and HCCs per animal.

The animals receiving 5.0 g/L TCA had significantly ($P<0.001$) reduced body weights and significantly increased ($P<0.01$) liver weights, irrespective of ENU-initiation. The liver weights of the 2.0 g/L TCA group were significantly increased ($P<0.001$); however, their body weights were unaffected. The increased liver weight in this group was attributed to an increased incidence in tumors. The kidney weights in the high-dose groups were significantly decreased ($P<0.001$).

TCA increased the incidence of animals with adenomas and HCCs and the number of adenomas and HCCs per animal ($P<0.001$) compared to the negative control group. Table 8 summarizes the incidence of adenomas and HCC observed in the test animals. These increases did not appear to be dose-dependent. ENU did not effect the ability of TCA to induce hepatocarcinogenesis. The authors concluded that TCA acted as a complete hepatocarcinogen in mice. [Herren-Freund and Pereira, 1987; Herren-Freund *et al.*, 1987].

Table 8: Incidence of Adenomas and Hepatocellular Carcinomas in Mice Treated with Trichloroacetic Acid

(ENU/Promoter) ($\mu\text{g/g}$) (g/l)	Animals with Adenomas (%)	No. of Adenomas Per Animal ^a	Animals with Carcinomas (%)	No. of Carcinomas Per Animal ^b
TCA (10.0/5)	11 (39%)	0.61 \pm 0.16	15 (54%)	0.93 \pm 0.22
TCA 2.5/5	6 (26%)*	0.30 \pm 0.12*	11 (48%)*	0.57 \pm 0.21*
TCA 2.5/2	11 (33%)*	0.42 \pm 0.12*	16 (48%)*	0.64 \pm 0.14*
TCA 0/5	8 (36%)*	0.50 \pm 0.16*	7 (32%)*	0.50 \pm 0.17*
NaCl 10.0/2	9 (39%)	0.52 \pm 0.15	9 (39%)	0.57 \pm 0.20
NaCl 2.5/2	1 (5%)	0.05 \pm 0.05	1 (5%)	0.05 \pm 0.05
NaCl 0/2	2 (9%)	0.09 \pm 0.06	0 (0%)	0.00
NaCl 2.5/500	1 (6%)	0.06 \pm 0.06	0 (0%)	0.00
NaCl 0/500	2 (9%)	0.09 \pm 0.06	0 (0%)	0.00

* indicates the results are significantly ($p < 0.001$) different from the appropriate NaCl control group.
a,b mean \pm standard deviation

reference: Herren-Freund *et al.*, 1987

- ***dermal mice :***

TCA was one of the compounds used in a study to determine the influence of scarification or preliminary treatment of the skin with chemicals that produce hyperplasia or inflammation on the initiation of tumors by urethane. Four groups of 20 Hall strain male mice received a single application of 5% TCA in 0.25 ml acetone solution to the skin on the right side of the back at intervals of 1, 2, 3 or 5 days before the ingestion of 25 mg of urethane. Seven days after receiving the urethane injection, then every seventh day for 20 weeks, the entire area of the animals' backs were treated by skin painting with croton oil in acetone. At the time of each painting, and every two weeks after week 16, the number of survivors, the number of papillomata, and their distribution on the skin of the back were recorded. The final count was made two weeks after the last application of croton oil.

Treatment with TCA produced considerable thickening and scaliness. Two weeks after the TCA treatment, some of the mice reportedly had some ulceration, but no scarring was noted. Preliminary treatment with TCA significantly increased (P value not reported), the incidence of urethane-induced tumors in mice [Pound and Withers, 1963].

- **dermal, Triturus cristatus:**

TCA was used as a noncarcinogenic, irritating control in a study of the induction and differentiation of epithelial tumors in newts, strain *Triturus cristatus*. A group of 20 newts were administered a single, injection (0.01-0.02 ml) of TCA in 2% aqueous solution on the dorsal, lateral, and ventral trunk, the neck pouch, and the tail. The animals were observed for epithelial tumors for 1 year. They were randomly sacrificed at various times during the study between 1 week and 1 year after injection. Complete necropsies were performed at the time of sacrifice.

TCA was reported to be exceptionally irritating, all of the newts had inflammatory reactions which subsided within 5-10 days. Histological examinations showed no evidence of tumors, only thickening of the epithelium. It was reported that 16 of the 20 animals treated with TCA died after treatment of an unspecified time point. TCA was determined to not be carcinogenic [Seilern-Aspang and Kratochwil, 1962].

E. Reproductive Effects and Teratogenicity

1. Case Reports

No data were found in the literature on the reproductive or teratogenic effects of either DCA or TCA in humans.

2. Animal Data

a) Dichloroacetic Acid

- **oral, rats:**

In an abstract by Smith *et al.*, it was reported that DCA is teratogenic in rats. Pregnant Long-Evans rats were given daily oral (via intubation) doses of the compound at concentrations of 0 (vehicle control), 900.0, 1400.0, 1900.0, or 2400.0 mg/kg/day on days 6-15 of gestation. Control groups were administered distilled water. On day 20 of the study, the animals were sacrificed and necropsied. Clinical signs, weight change, organ weights and uterine contents were evaluated. Live fetuses were examined for external, skeletal, and soft tissue malformations.

During the treatment period, 8 dams died; 1/14 in the 1400.0 mg/kg dose group, 3/20 in the 1900.0 mg/kg group, and 4/21 in the highest dose group (2400.0 mg/kg). Reduction in maternal weight gain was observed in all of the treated animals. Increased spleen, kidney, and liver weights were found to be dose-related and the percentage of resorbed implants per litter was significantly elevated at all doses.

Dose-dependent reductions in weight and length were observed in all of the live fetuses. The mean frequency of soft tissue malformations ranged from 10% (@ 900.0 mg/kg) to 73% (@ 2400.0 mg/kg). Of the malformations observed, cardiovascular system malformations (defects between the ascending aorta and right ventricle) were predominant. There were no skeletal malformations observed. The authors concluded that DCA was teratogenic in rats at all doses tested [Smith *et al.*, 1989a].

- **oral rats :**

As described in an abstract by Epstein *et al.*, an investigation was performed to determine the relationship between a single maternal dose of DCA and fetal cardiogenic outcome and to characterize the morphology of DCA-induced fetal heart defect. An unspecified number of pregnant Long-Evans rats were dosed by oral intubation with 0, 1900.0, 2400.0, or 3500.0 mg/kg DCA in an unspecified vehicle on days 6-15 of gestation. Maternal parameters were observed and recorded until day 20 when the animals were sacrificed and necropsied. The fetuses were evaluated for gross and microscopic abnormalities.

The incidence of heart defects was greatest following exposure to DCA on days 10 and 12 of gestation. Retention of embryologic communication between the aorta and right ventricle was observed. These observations suggest that DCA interferes with the closure of the secondary and/or tertiary interventricular foramina. No other data were reported [Epstein *et al.*, 1990].

b) Trichloroacetic Acid

- **oral rats :**

The teratogenic effects of TCA were investigated using female Long-Evans hooded rats. In this study, maternal toxicity (decreases in body weight, increases in liver, kidney, and spleen weights, and premature death), embryoletality, fetal weight and lengths, and fetal malformations (external and internal) were evaluated. Pregnant rats were administered daily oral doses of 0 (vehicle control), 330, 800, 1200.0, or 1800.0 mg/kg TCA dissolved in water for 10 consecutive days (days 6-15 of gestation). Distilled water was given to a control group. On the 20th day of gestation, the dams were sacrificed and both the dams and fetuses were examined.

All of the dams (except 1 accidentally killed) survived to the scheduled termination on gestational day 20. No overt signs of maternal toxicity were observed. Pregnancy rates were high and equivalent in all of the groups. Analysis of the average percent maternal weight gain adjusted for gravid uterine weight from days 0-20 revealed no significant differences from the control. The administration of this compound did have somewhat pronounced effects on the dams in the form of body and organ weight changes, at doses that produced *in utero* toxicity (fetal death, weight reduction, and malformations). The 330 mg/kg dose group had significantly lower body weights than the controls at both day 0 and 20. The change in weight from days 6-9 and 15-20 was significantly reduced in the dams receiving 800.0 ($P \leq 0.007$), 1200.0 ($P \leq 0.001$), and 1800.0 ($P \leq 0.0004$) mg/kg TCA. The maternal liver weights were unaffected by TCA; however, spleen and kidney weights increased significantly ($P = 0.0001$) in a dose-dependant manner in comparison to the control group. The total number of implants per litter also showed a dose-related increase ($P = 0.0004$) and was significantly higher than the control at doses of 800.0 mg/kg and above. Table 9 summarizes the maternal toxicity observed.

Treatment with TCA produced a substantial increase in embryoletality at 800.0 ($P = 0.003$), 1200.0 ($P < 0.0001$), and 1800.0 ($P < 0.0001$) mg/kg TCA with 35, 62, and 90% of implants resorbed, respectively. At 1200.0 and 1800.0 mg/kg, 5 and 12 litters were totally resorbed, respectively. The fetal weight and crown-rump lengths were significantly less in fetuses whose mothers received TCA than in fetuses of control animals. There were no difference in the rates of external malformations between the control and treated animals; however, total soft tissue malformations were significantly increased in all of the treated animals compared to the controls (330.0 mg/kg, $P = 0.029$; 800.0-1800.0 mg/kg, $P < 0.0001$). These malformations occurred in the cardiovascular system. Skeletal malformations, particularly around the eye were also observed. Orbital malformations, the second largest category of malformation, were significantly increased at 1200.0 mg/kg ($P = 0.0012$) and 1800.0 mg/kg ($P < 0.0001$). An increasing dose-related response was found for external ($P < 0.007$), total soft tissue ($P = 0.0001$), cardiovascular ($P = 0.0001$), skeletal ($P = 0.0001$), and orbital ($P < 0.0001$) malformations. Table 10 summarizes the fetal malformations observed.

The authors concluded that TCA administered orally to rats early in organogenesis causes systemic toxicity in the dams and embryonic death and malformation in the fetuses [Smith *et al.*, 1989b; Smith *et al.*, 1988].

TABLE 9: Reproductive Performance and Maternal Observations at Sacrifice Following Exposure to Trichloroacetic Acid in Rats

<u>Observation</u>	<u>Treatment (Number of Sperm Positive Females)</u>				
	<u>Control (26)</u>	<u>TCA 330 (21)</u>	<u>TCA 800 (20)</u>	<u>TCA 1200 (21)</u>	<u>TCA 1800 (20)</u>
Viable Litters	26	19	17	14*	8*
Litters Totally	0	0	1	5*	12*
Resorbed					
Mean Implants Per Litter	12.5 ± 1.36	12.6 ± 3.39	13.2 ± 1.26*	13.7 ± 1.67*	13.7 ± 2.62*
Mean Live Fetuses Per Litter	10.9 ± 2.30	11.6 ± 3.49	8.7 ± 4.10	5.1 ± 4.29*	1.5 ± 2.44*

*Significantly different from control vehicle, P ≤ 0.05.

reference: Smith *et al.*, 1989b.

TABLE 10: Incidence of Fetal Anomalies Following Exposure to Trichloroacetic Acid on Days 6-15 of Gestation in Rats ¹

	<u>Treatment (mg/kg/day)</u>				
	<u>Water</u>	<u>TCA (330.0)</u>	<u>TCA (800.0)</u>	<u>TCA (1,200.0)</u>	<u>TCA (1,800.0)</u>
<u>Malformations²</u>					
External	0.64 ± 3.3 (1)	0	2.63 ± 7.0 (3)	11.73 ± 28.8 (3)	7.81 ± 17.6 (2)
Total soft tissue	3.50 ± 8.7 (4)	9.06 ± 12.9*	30.37 ± 28.1*	55.36 ± 36.1*	96.88 ± 8.8*
Cardiovascular	0.96 ± 4.9 (1)	5.44 ± 10.0*	23.59 ± 28.0*	46.83 ± 36.5*	94.79 ± 9.9*
Urogenital	3.02 ± 8.6 (3)	3.63 ± 8.1 (4)	4.17 ± 9.0 (4)	7.14 ± 26.7 (1)	3.13 ± 8.8 (1)
Skeletal	0	0	0	26.39 ± 41.1* (4)	50.00 ± 44.7* (4)
Orbital ³	0	0	2.63 ± 6.9 (3)	14.65 ± 28.2* (5)	21.87 ± 30.5* (4)

¹Mean ± SD % fetuses affected per litter, denominator was number of fetuses examined (number of litters affected).

²Soft tissue and skeletal combined.

³Numbers in parenthesis indicate number of litters examined.

*Significantly different from control P ≤ 0.05.

reference: Smith *et al.*, 1989b

F. Genetic Toxicology

1. Prokaryotic Data

a) Dichloroacetic Acid/Trichloroacetic Acid

- ***Salmonella typhimurium*:**

In the standard Ames test, both DCA and TCA were not found to be mutagenic in *Salmonella typhimurium* strains TA98 and TA100. No other data were reported [Voogd *et al.*, 1989].

- ***Salmonella typhimurium*:**

The mutagenic effects of DCA and TCA, extracted from humic substances, were investigated using *Salmonella typhimurium* strain TA100 with and without S-9 activation. Both DCA and TCA were found to be nonmutagenic in *Salmonella*. No other data were reported [Sato *et al.*, 1985].

- ***Salmonella typhimurium*:**

The genotoxicity of TCA and DCA in *Salmonella typhimurium* was determined by the *umu*-test. DCA and TCA (0.2 ml) were added with and without metabolic activation to *Salmonella* strain TA1535/pSK1002 for a 2-hour incubation period. Both compounds were found to be genotoxic with microsomal activation at a minimum dose of 58.5 µg/ml/OD₆₀₀ (cell density before assay). TCA was weakly positive at this same concentration in the absence of metabolic activation [Ono *et al.*, 1991].

- ***Salmonella typhimurium*:**

The mutagenic effects of TCA and DCA were determined in the *Salmonella* histidine reverse mutation assay using *Salmonella typhimurium his* - strains TA98 and TA100 with and without metabolic activation from rat liver S9. The authors concluded that DCA and TCA were nonmutagenic in all strains of *Salmonella* under all test conditions [Kopfler *et al.*, 1985].

- ***Klebsiella pneumoniae*:**

The mutagenic activity of DCA and TCA was tested in the fluctuation test using *Klebsiella pneumoniae*. Neither compound expressed any mutagenic effects toward the *Klebsiella* in this study. No other data were reported [Voogd *et al.*, 1989].

b) Dichloroacetic Acid

- **Salmonella typhimurium:**

In an unpublished NTP study, DCA was tested in a preincubation modification of the *Salmonella* assay in the presence and absence of activation (from Arochlor 1254-induced Sprague-Dawley rats and Syrian hamsters) in strains TA 98, TA1535, and TA100. DCA was tested in dimethyl sulfoxide at concentrations ranging from 0.0 µg/plate-6666.0 µg/plate. DCA was found to be positive in TA100 and TA1535 in the absence of metabolic activation and negative in TA98 in the presence and absence of activation [NTP, 1991; NTP/NIEHS, 1991].

- **Salmonella typhimurium:**

In the DNA-repair test, 31 mg of DCA was found to be nonmutagenic in *Salmonella typhimurium* strains TA1950 uvrB, TA2322 polA, TS24 recA, and hisG r. No other data were reported [Waskell, 1978].

c) Trichloroacetic Acid

- **Salmonella typhimurium:**

In the Ames test, the mutagenic effects of TCA were tested in *Salmonella typhimurium* strains TA100 and TA98 with and without metabolic activation. TCA was tested at concentrations ranging from 0.0-5000.0 µg/plate. No mutagenic effects were observed [Moriya *et al.*, 1983].

- **Salmonella typhimurium:**

In the standard Ames test, TCA was found to be nonmutagenic in *Salmonella typhimurium* strains TA98, TA100, and TA200 with and without metabolic activation. The maximum nontoxic quantity of TCA tested was 0.45 mg/plate [Waskell, 1978].

- **Salmonella typhimurium:**

The mutagenic effects of TCA were tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1538. TCA was nonmutagenic in all strains of *Salmonella* tested. No other data were reported [Ortali *et al.*, 1977].

- **Salmonella typhimurium:**

TCA was 1 of 93 compounds suspected to be a reaction product from the chlorination process of groundwater and tested to determine its mutagenic effects. TCA was tested in the standard Ames test using a single *Salmonella typhimurium* strain, TA100. This compound was found to be nonmutagenic to *Salmonella* at concentrations of 10³, 10², 10¹, 10⁰, and 10⁻¹ µg per plate [Rapson *et al.*, 1980].

- **Salmonella typhimurium:**

TCA was not found to be mutagenic *Salmonella typhimurium* strains TA1535, TA100, TA1538, and TA98. No other data were reported [Bignami *et al.*, 1977].

- **Salmonella typhimurium:**

The mutagenicity of TCA was evaluated with eight histidine-requiring mutants of *Salmonella typhimurium* by measuring the frequency of reversion to histidine independence. TCA was found to be nonmutagenic as unspecified concentrations to all strains tested. No other data were reported [Andersen *et al.*, 1972].

- **Streptomyces coelicor:**

The mutagenic effects of TCA were studied in *Streptomyces coelicor* strain (073) his A1. TCA was determined to be nonmutagenic. No other data were reported [Ortali *et al.*, 1977].

2. Eukaryotic Data

a) Dichloroacetic Acid

No data were found in the literature on the genotoxic effects of DCA in eukaryotic organisms.

b) Trichloroacetic Acid

- **T₄ bacteriophage:**

The mutagenic potential of TCA was examined in two tests using T₄ bacteriophage. In the first study, *Escherichia coli* B was cultured with the phage particles and 100 µg TCA/plate (unreported concentrations), and the resulting number of rII mutant plaques of T₄ bacteriophage were counted. Under these conditions, TCA was nonmutagenic. In the second study, *E. coli* B was incubated with 100 µg TCA/plate and the rII mutant of T₄ bacteriophage, Ap72, to detect reversions from the mutant to the wild type. TCA did not induce a significant number of reversions, and was considered nonmutagenic [Andersen *et al.*, 1977].

- **Aspergillus nidulans:**

It was determined that TCA does not cause the induction of point mutations and somatic segregation in *Aspergillus nidulans* using the spot test technique. No other data were reported [Bignami *et al.*, 1977].

- **bone marrow aberration assay :**

The cytogenetic effect of TCA on somatic cells was studied using a bone marrow aberration assay. Groups of adult Swiss mice were administered TCA in distilled water either intraperitoneally (i.p.) or orally (p.o.). Negative control groups were administered distilled water. The following variations were utilized in this study: a) An unspecified number of animals were injected i.p. with 500.0 mg/kg of test compound and sacrificed after 6, 24, or 48 hours, b) An unspecified number of animals were injected i.p. with 125.0, 250.0, or 500.0 mg/kg of TCA and sacrificed after 24 hours, c) An unspecified number of animals were tested with 500.0 mg/kg either i.p. or p.o., and were sacrificed after 24 hours, d) An unspecified number of animals were administered a total of five 100.0 mg/kg doses every 24 hours and were sacrificed 24 hours after the last injection. To score the aberrations, one hundred good metaphase spreads were examined for each animal.

Chromosomal aberrations such as chromatid gaps and breaks (including fragments), isochromatid gaps, chromatid exchanges, rings and double minutes were recorded in every treatment group. Usually 1 or 2 aberrations occurred in a cell if there were any. The highest percentage of aberrations (7.00 ± 2.00) was produced by the highest dose 24 hours after treatment. Relatively fewer effects were observed 6 and 48 hours after treatment. Table 11 summarizes the frequency of chromosomal aberrations in mice treated i.p. Regarding route sensitivity, the effects were higher when the chemical was administered i.p. rather than p.o.. Table 12 summarizes the route-dependent aberrations. The cytogenetic effect induced by repeated treatment with fractionated doses was less than the effect induced by the equivalent dose administered at one time. The authors concluded that TCA is capable of causing cytogenetic mutations in somatic cells [Bhunya and Behera, 1987].

Table 11: Frequency of Chromosomal Aberrations in Bone-Marrow Cells of Mice Treated (ip) with TCA

	Dose (mg/kg)						
	<u>500</u>	<u>500</u>	<u>500</u>	<u>250</u>	<u>125</u>	<u>5 x 100 (chronic)</u>	<u>Composite Control</u>
Hours after Treatment	6	24	48	24	24	120	---
Number of Cells Studied	300	300	300	300	300	300	1000
Chromatid gaps	10	12	4	9	3	4	5
Isochromatid gaps	---	1	2	3	---	---	---
Chromatid breaks	9	13	10	12	13	5	2
Chromatid exchange	---	---	---	---	1	1	---
Rings	---	1	---	---	2	1	---
Double Minutes	---	7	1	1	2	2	---
Total without gaps	9 (19)	21 (34)	11 (17)	13 (25)	18 (21)	9 (13)	2 (7)
% of aberrations without gaps (\pm S.E.)	3.00 \pm 1.15 (6.33 \pm 1.20)	7.00 \pm 2.00 (11.33 \pm 2.40)	3.66 \pm 0.66 (5.66 \pm .88)	4.33 \pm 1.20 (8.33 \pm 0.33)	6.00 \pm 1.15 (7.00 \pm 1.52)	3.00 \pm 0.57 (4.33 \pm 0.87)	0.20 \pm 0.13 (0.70 \pm 0.26)
% of aberrant metaphases without gaps	2.66 (5.66)	6.00 (10.00)	3.66 (5.00)	3.33 (6.66)	2.33 (3.33)	2.33 (3.33)	0.20 (0.70)

Results in parentheses are with gaps
Significant difference from control ($P \geq 1.96$)

reference: Bhunya and Behera, 1987

Table 12: Route-Dependent Chromosome Aberrations Induced by TCA in the Bone-Marrow Cells of Mice

	ROUTE			
	<u>Intraperitoneal</u>	<u>Control</u>	<u>Oral</u>	<u>Control</u>
Hours after treatment	24		24	
Number of cells studied	300	1000	300	600
Chromatid gaps	12	5	6	3
Isochromatid gaps	1	---	1	---
Chromatid breaks	13	2	9	1
Rings	1	---	---	---
Double Minutes (DM)	7	---	2	---
Total without gaps	21 (54)	2 (7)	11 (18)	1 (4)
% of Aberrations	7.00±2.00	0.20±13	3.66±0.33	0.16±0.16
without gaps ± S.E.	(11.33±2.40)	(0.70±0.26)	(6.00±1.52)	(0.66±0.33)
Z Value	7.83 (9.24)	---	4.31 (4.88)	---
% of Aberrant metaphases	6.00 (10.00)	0.20 (0.70)	1.66 (2.66)	0.16 (0.66)
without gaps				

Results in parentheses are with gaps.

Significant difference from control ($P \geq 1.96$).

reference: Bhunya and Behera, 1987

- ***micronucleus assay :***

The micronucleus assay was used to determine whether TCA is genotoxic in mouse somatic cells. Groups of 3 Swiss mice were administered two intraperitoneal injections, at 24 hour intervals, of TCA in distilled water at doses of 125.0, 250.0, or 500.0 mg/kg. Control groups were administered distilled water only. Six hours after the second injection, the animals were sacrificed, and 1000 polychromatic erythrocytes (PECs), normochromatic erythrocytes (NCEs) and nucleated cells were examined per animal for the presence of micronucleus cells (MNs). All dose levels produced a significant increase in MNs, PCEs, and NCEs compared to the controls. In nucleated cells, an increase was only noted at the highest dose level. The MN were either dot- or ring- shaped and ranged from one fifth to one ninth of the cell size in the different cell types. The frequency of MN was highest in PCEs and lowest in nucleated cells. The authors concluded that TCA is genotoxic [Bhunya and Behera, 1987].

- **sperm-head abnormality assay:**

The cytogenetic effects of TCA on germ line cells of the mouse were determined using the sperm-head abnormality assay. Groups of three male Swiss mice were administered doses of 125.0, 250.0, or 500.0 mg/kg of the TCA in distilled water intraperitoneally. Each dose was divided into 5 equal parts with each part successively administered intraperitoneally every 24 hours. The animals were sacrificed 35 days after the last injection. Five hundred sperms per animal were collected and scored for abnormalities. TCA induced a variety of abnormal sperm-heads including double headed, giant size, calyx, round, spear, banana, and amorphous types. The highest frequency of sperm-head abnormalities was induced by the highest dose (500.0 mg/kg). Table 13 summarizes the frequency at which sperm-head abnormalities occurred. The authors concluded that TCA is capable of producing genotoxicity in the sperm-head abnormality assay [Bhunya and Behera, 1987].

Table 13: TCA-Induced Sperm-Head Abnormalities in Mice

	<u>Dose (mg/kg)</u>			
	<u>125</u>	<u>250</u>	<u>500</u>	<u>Control</u>
Nos. of Animals	3	3	3	3
Days after treatment	35	35	35	35
Tot. Nos. of Sperm Studied	1500	1500	1500	3000
Tot. Nos. of Abnormal Sperm	66	67	114	62
Percentage of Abnormal Sperm ± SE	4.40±0.11	4.40±0.76	7.60±0.60	2.06±0.08

Significant difference from control ($P \geq 1.96$)

reference: Bhunya and Behera, 1987

3. Plant Data

- ***Arabidopsis:***

The mutagenic effects of TCA and dimethyl sulfonate (DMS), alone and combined, were studied in *Arabidopsis* of the *Estland* family. *Arabidopsis* seeds were treated with an aqueous TCA solution at concentrations of 0.055 and 0.1%, and with a combination of TCA (at the same concentration) and 0.15% DMS solution. The seeds were planted and the second generation plants (M2) were used to examine chlorophyll mutations. The frequency of chlorophyll mutations in these plants was increased TCA treatment, but decreased by TCA/DMS treatment. When the seeds were exposed to 0.05% TCA, 0.1% TCA, 0.05% TCA/0.15% DMS, and 0.1% TCA/0.15% TCA/DMS, the mutant frequencies in the M2 generation were $2.84 \pm 1.25\%$, $5.38 \pm 0.55\%$, $2.05 \pm 0.33\%$, and $2.43 \pm 0.30\%$, respectively. These compare to a control frequency of $2.68 \pm 0.52\%$. The authors hypothesized that TCA increases gene "productivity" by some nonspecific gene activation. The synthesis of proteins then becomes uncontrolled, and ultimately leads to stimulation (and mutation). The authors also concluded that histones play an important role in the mutation process [Plotnikov and Petrov, 1976].

- ***Faba vulgaris:***

The cytological effects of TCA were determined in *Faba vulgaris*. *Vulgaris* seeds were germinated and treated with a concentration of 3.1×10^{-4} M TCA. After 6 days of growth the root systems were fixed. Controls received no treatment. The results obtained in this study indicate that TCA is capable of causing mitotic inhibition and chromosomal aberrations. The authors proposed that the mode of production of aberrations by TCA involves its effect on the precipitating action of proteins. TCA is also capable of disturbing the mitotic cycle in *vulgaris* by inducing fast mitosis with a long interphase. The author suggests that TCA not be used on *vulgaris* [El-Sadek, 1972].

G. Other Toxicological Effects

1. Immunotoxicity

- **oral, rats:**

The effects of DCA and TCA on several immunological parameters were assessed in a 90-day study described in section V.C.2. Male Sprague-Dawley rats were divided into four treatment groups (per test compound) with 10 animals per group and administered DCA and TCA in their drinking water at concentrations of 0, 50.0, 500.0, or 5000.0 for 90 days. Following the exposure period, the following immunological parameters were assessed: T cell-dependent IgG antibody production, delayed-type hypersensitivity, natural killer cell cytotoxicity, and production of macrophage-derived prostaglandin E2 and lymphocyte-derived interleukin 2. No evidence of DCA or TCA related changes were observed for any of the parameters evaluated [Mather *et al.*, 1990].

2. Neurotoxicity

No data were found in the literature on the neurotoxicity of either DCA or TCA.

3. Biochemical Toxicology

General Biochemistry

a) Dichloroacetic Acid/Trichloroacetic Acid

- **oral, rats**

As part of a study examining TCA- and DCA-induced single-strand breaks in hepatic DNA (see Section V.G.3., single strand break induction studies), the effect of TCA and DCA on serum enzyme levels was examined in male Sprague-Dawley rats. (This effect was also tested in mice, see below.) Groups of 4 rats were given an oral dose of either TCA (3.1 mmol/kg) or DCA (3.8 mmol/kg) as a 1% aqueous solution of Tween 80 in distilled water (total volume of 1 ml). Five control animals received an equivalent volume of vehicle. Twenty-four hours after dosing, the animals were sacrificed and blood samples were taken from the inferior vena cava. Serum aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured in each sample. The results show that treatment with TCA or DCA did not cause a significant difference in AST and ALT levels when compared to controls ($P < 0.05$) [Nelson, 1989a].

- oral, mice

As part of a study examining TCA- and DCA-induced single-strand breaks in hepatic DNA (see Section V.G.3., single strand break induction studies), the effect of TCA and DCA on serum enzyme levels was examined in male B6C3F1 mice. (This effect was also tested in rats, see above.) Groups of 5 mice were given an oral dose of either TCA (3.1 mmol/kg) or DCA (3.9 mmol/kg) as a 1% aqueous solution of Tween 80 in distilled water (total volume of 1 ml). Five control animals received an equivalent volume of vehicle. Twenty-four hours after dosing, the animals were sacrificed and blood samples were taken from the inferior vena cava. Serum aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured in each sample. The results show that treatment with TCA or DCA did not cause a significant difference in AST and ALT levels when compared to controls ($P < 0.05$) [Nelson, 1989].

- in vitro, rats:

The *in vitro* interactions of DCA and TCA with rat liver glutathione S-transferases (GST) were studied using glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates. To measure the inhibition of GST by TCA and DCA, crude supernatants of rat liver were exposed to the test compounds at concentrations of 2.5, 10.0, 25.0 or 50.0 mM in an enzyme reaction mixture containing CDNB and GSH (at a concentration of 0.5 mM). GST activity was measured following exposure. In addition, the inhibition of the different GST isoenzymes (M, E, C, B, A and AA) was measured by adding 12.5 mM of the test compound to the reaction mixture containing CDNB and GSH (at a concentration of 1 mM). Enzyme kinetic experiments were conducted using the separated GST isoenzymes with CDNB and GSH as the substrates, and GSH titration experiments were performed (details of these procedures were not reported).

Inhibition of GST in crude rat liver supernatants was observed to be dose-dependant, but not linear. Since neither TCA nor DCA caused protein precipitation, the decreased GST activity was not attributed to this phenomenon. Each of the GST isoenzymes was also inhibited by 12.5 mM TCA and DCA, but to different degrees. For example, GST M was inhibited more by TCA than GST AA, and GST A was inhibited by DCA to a greater extent than GST B. Kinetic studies did not reveal competitive inhibition with any of the six different GST isoenzymes using either substrate. According to the authors, this indicates that TCA- and DCA-induced inhibition of GST activity is not due to their competition with CDNB or GSH as a substrate for GST. Titration of the GSH remaining after incubation with TCA or DCA revealed that no conjugate formation had occurred. The authors concluded that TCA and DCA interact with GST by direct binding, and that GST could have a protective function against these compounds [Dierickx, 1984].

- ***in vitro, mice:***

The ability DCA and TCA to increase hexose transport was investigated in Swiss 3T3 mouse fibroblast cells and was compared to the effects of insulin. The cultured cells were incubated in the presence and absence of intra- and extracellular Mg^{2+} and Ca^{2+} with 250 μM of DCA or TCA for 2 hours. The uptake of hexose was measured. It was observed that DCA significantly ($P < 0.01$) stimulated hexose transport activity, while TCA had no significant effect. The authors reported the effect of DCA to be dose- and time- dependent with half-maxima at 40 minutes and 100 μM ; however, the protocol only reported one time-point and one dose. The results also show that the removal of intra- and extracellular Mg^{2+} and Ca^{2+} with ethylenediaminetetraacetic acid (EDTA) suppressed hexose transport activity, while removal of extracellular Mg^{2+} and Ca^{2+} had no effect. Further studies were done to determine whether the inhibition of hexose transport was due to the cytotoxicity of DCA or to the removal of Ca^{2+} or Mg^{2+} . These compounds were reintroduced, individually, to the medium. The inhibitory effect of these agents was removed by restoration of Mg^{2+} to the culture medium. The authors stated that the results of this study show that DCA has an insulin-like stimulatory effect on hexose transport and that Mg^{2+} may be involved in the transmission of the signal of this compound which induces hexose transport in Swiss 3T3 cells [Kitagawa *et al.*, 1987].

- ***in vitro, sheep:***

The ability of TCA and DCA to cause oxidant stress in erythrocytes was studied using the blood from Dorset sheep with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. The oxidant stress was measured by changes in methemoglobin (METHB) formation and by reduced glutathione (GSH) levels. Blood from 10 nonpregnant female Dorset sheep was collected and divided into 3 test groups treated with either TCA, DCA, or equal amounts of both TCA and DCA. Each chemical was tested at concentrations of 0 (no treatment negative control), 0.1, 1.0, and 10.0 mM/ml of blood. Blood samples were incubated with the test compound for 2 hours at 37°C, and the hematological parameters (METHB formation and GSH levels) were measured.

Significant dose-dependent increases in METHB were seen following treatment with TCA, DCA ($P < 0.001$), and a combination of the two compounds ($P < 0.01$). Further analysis revealed a linear trend in each of these test groups ($P < 0.002$ for TCA, $P < 0.001$ for DCA, and $P < 0.02$ for both). Using Turkey's multiple range test, it was determined that METHB formation following treatment with 0.1 and 1.0 mM TCA was not significantly different. However, 10.0 mM of TCA significantly increased ($P < 0.05$) the percentage of METHB when compared to the other dose groups. Treatment with 10.0 mM DCA caused a significantly higher ($P < 0.05$) formation of METHB when compared to treatment with 0.1 mM DCA or to the control group. METHB formation was increased by 5.1%, 3.3%, and 6.8% following treatment with TCA, DCA, or a combination of the two, respectively.

Treatment with TCA, DCA, and a combination of both compounds significantly effected (decreased) the level of GSH ($P=0.017$, $P=0.01$, and $P=0.002$, respectively). However, linear trends were observed in the TCA test group ($P=0.57$) and in the combination test group ($P<0.02$), but not in the DCA test group. The Tukey multiple range test revealed that treatment with 10.0 mM TCA caused a significant decrease ($P<0.05$) in GSH levels when compared to other doses of TCA; these other dose groups did not differ among themselves. Also, GSH levels following treatment with 0.1 mM DCA were significantly different ($P<0.05$) than the levels measured after DCA treatment at other doses. Each dose of the combination of TCA/DCA resulted in GSH levels that were significantly lower ($P<0.05$) than the control; however, the levels within each dose group did not differ significantly from each other.

The results indicate that TCA and DCA are capable of causing oxidative stress in G-6-PD-deficient red blood cells of Dorset sheep. It was observed that TCA and DCA influenced the formation of METHB, in a dose-dependent fashion, and mildly influenced the reduction of GSH. The combined TCA/DCA treatments resulted in a less than additive response for METHB and no discernible interaction for GSH effects. The authors state that although G-6-PD-deficient erythrocytes in sheep are similar to the G-6-PD-deficient erythrocytes in humans, they respond with less sensitivity to a variety of oxidant stressor agents. Consequently, it is not unreasonable to hypothesize that human G-6-PD-deficient erythrocytes may be somewhat more sensitive than the sheep erythrocytes to DCA and TCA [Calabrese and Leonard, 1984].

b) Dichloroacetic Acid

• oral rats/in vitro, rats

The effect of DCA on rat hepatic mRNA was examined *in vivo* using male Sprague-Dawley rats and *in vitro* using isolated rat hepatocytes. The mRNA responses induced by carbohydrate feeding *in vivo*, and by glucose administration to hepatocytes in culture, were compared with the mRNA responses induced by DCA both *in vivo* and in culture. First, in two separate experiments, groups of four rats were given feed containing DCA (neutralized with NaOH) at a concentration of 100 mg DCA/100 gm body weight. A fat-free 60% sucrose diet was offered to a second group of rats, and a control group was given standard chow. All rats were treated for six days, sacrificed, and evaluated for changes in body weight, liver weight, blood glucose levels, and malic enzyme (ME) activity. In addition, RNA was isolated from the liver of each animal, and mRNA activity profiles were evaluated to gain further insights into the mechanism of DCA induction of ME. In the second part of the study, isolated rat hepatocytes were treated with 5.5 mM glucose (control), 27.5 mmol/L glucose, or 10 mM DCA with 5.5 mmol/L glucose. After 6 days, the cells were harvested, RNA was isolated, and mRNA activity profiles were produced using two-dimensional gel autoradiogram.

Rats administered DCA did not grow as large as the control animals; however, liver weight in DCA-treated animals (8.5 ± 0.3 g) was significantly greater ($P < 0.05$) than in controls (6.7 ± 0.2 g). The results from both experiments show that administration of DCA significantly increased ($P < 0.05$) ME activity in rats. *In vivo*, DCA feeding also induced changes in hepatic mRNA that paralleled the changes produced by *in vivo* carbohydrate (glucose) feeding; six of the nine "spots" (on the two-dimensional gel autoradiogram) that changed were changed significantly ($P < 0.05$) from controls and in similar directions by both carbohydrate feeding and DCA administration. The results of the *in vitro* study support these findings. Six of the seven spots changed by *in vivo* carbohydrate feeding also changed in response to glucose addition to the isolated hepatocytes. The addition of 10.0 mM DCA to hepatocyte cultures significantly increased 4/7 spots induced by DCA *in vivo* and changed by glucose treatment *in vivo* and *in vitro*. The mRNA for ME was among those mRNA sequences induced by glucose and DCA. Examination of the pyruvate concentrations in the culture media gave further information about the mechanism of DCA regulation of gene expression. Glucose feeding led to enhanced pyruvate concentrations, and DCA exposure led to reduced pyruvate concentrations when compared to controls.

The authors stated that the flux of pyruvate through pyruvate dehydrogenase generates the signal leading to the alteration in the various mRNA species. Also, the marked overlap in mRNA response to both carbohydrate and DCA suggests that DCA acts by a similar mechanism to that of carbohydrate feeding, and that the signal regulating the content of the carbohydrate responsive mRNAs is derived from mitochondria pyruvate oxidation [Mariash and Schwartz, 1986].

- **oral mice:**

The effects of DCA on ornithine decarboxylase activity and hepatic growth in mice have been reported in an abstract of a proposed Environmental Protection Agency presentation. Groups of male B6C3F1 mice received doses of DCA in distilled drinking water at concentrations of 0, 0.5, and 5.0 g/L for 15 weeks.

During the first 4 weeks of exposure, liver weights of the animals in the 0.5 and 5.0 g/L dose groups had increased 28% and 119%, respectively, compared to controls and remained constant for the remainder of the study. After 2 weeks, the ^3H -Thy incorporation in the high dose mice was 50% that of the controls, and in the low dose mice the ^3H -Thy incorporation was 15% higher than control levels. At 4 weeks, ^3H -Thy incorporation was similar in all groups. At the conclusion of the study (15 weeks), low- and high-dose mice incorporated 45% and 67% more ^3H -Thy respectively, than control mice. No changes in hepatic ODC activity were observed. However, basal ODC activity in the kidney was observed to be 100 to 1000 times higher than the basal activity in the liver. The authors suggest that DCA may cause renal toxicity at high doses as ODC activity decreased 10-fold and 4-fold at 4 and 15 weeks, respectively. At 4 and 15 weeks, ODC activity in low-dose mice was observed to increase 4-fold and 3-fold, respectively. The

authors further suggest that DCA has no significant long-term effects on the induction of ODC, and that early liver growth following DCA exposure is predominantly hypertrophic [Potter *et al.*, 1990].

c) Trichloroacetic Acid

• oral, rats :

In conjunction with prechronic and chronic studies by Parnell, 1986 (see Section V.C.2 and V.D.2), potential carcinogenic effects of TCA were evaluated by measuring the induction of hepatic ornithine decarboxylase (ODC) activity in rat liver to determine whether a relationship exists between ODC activity and the promotion of cancer. Eight male Sprague-Dawley rats were administered, by gavage, a single 1500.0 mg/kg dose of TCA in distilled water. A control group (N=8) received distilled water only. Four animals from each group were sacrificed at either 5 or 18 hours after treatment, and liver samples were obtained and homogenized to determine the enzyme activity by measuring the release of radiolabeled carbon dioxide from DL-[1-¹⁴C]ornithine.

Five hours after treatment, ODC activity in the animals treated with TCA was significantly increased ($P \leq 0.005$) by 69.5 fold over the control animals. (The measured ODC activity in the treated animals was 56.96 ± 8.33 PMole ¹⁴CO₂/30 min/mg protein, and the activity of the control group was 0.82 ± 8.33 PMole ¹⁴CO₂/30 min/mg protein. After 18 hours, ODC activity was still significantly increased ($P \leq 0.001$) in treated animals (66.20 ± 6.96 PMole ¹⁴CO₂/30 min/mg protein) compared to the control group (2.54 ± 6.96 PMole ¹⁴CO₂/30 min/mg protein). The authors state that ODC induction has been associated with the promotion of skin and liver cancers and may be an enzymatic marker for tumor promotion. They continue that the results of this study did not provide information on the relationship between TCA's ability to induce hepatic ODC activity and its weak promoting activity [Parnell, 1986; Parnell *et al*, 1988].

• oral mice :

In a study to determine the synergistic effects of chlorinated compounds and 0.1% griseofulvin, TCA alone and combined with griseofulvin was given to mice in their drinking water to compare the effects of these compounds on porphyrin metabolism. Groups of five dd-y strain mice received 0.25% TCA, either alone or with 0.1% griseofulvin, in their drinking water. Untreated mice served as controls (n=5). All animals were sacrificed after receiving the treated water for 10-80 days. Body and liver weights were recorded, and liver and blood samples were analyzed for porphyrins (coproporphyrin, protoporphyrin, and uroporphyrin).

At the conclusion of the study, it was reported that the liver/body weight ratio of the animals receiving TCA only ($4.18 \pm 0.18\%$), was significantly lower ($P < 0.05$) than the ratio of the controls ($5.31 \pm 0.19\%$). The liver/body weight ratio in animals treated with TCA and griseofulvin did not change significantly. The TCA treated animals reportedly had enlarged livers. There was no evidence that TCA or TCA/griseofulvin had any effect upon porphyrin metabolism [Nonaka *et al.*, 1989].

Peroxisome Proliferation and Cytochrome Induction

- **oral, rats :**

In a carcinogenicity study described in section V.D.2, TCA's ability to induce peroxisomal proliferation was determined by measuring the oxidation of radiolabeled palmitoyl-CoA ($[1-^{14}C]$ palmitoyl-CoA). Fresh liver samples were collected from the male, 2/3 hepatectomized Sprague-Dawley rats treated in the initiation/promotion protocols previously described.

None of the animals treated with TCA using the induction protocol were observed to have any increased palmitoyl-CoA activities. In the promotion protocol, a significant ($P \leq 0.05$) increase in peroxisomal specific palmitoyl-CoA oxidation was observed in the TCA high dose group (5000.0 mg/L), during each testing period. The magnitude of the increase over controls was 10-20%.

The authors state that these results indicate that TCA is not a strong hepatic peroxisome inducer as the magnitude of the increase in peroxisomal proliferation observed in this study was small when compared to increases associated with "typical" peroxisomal proliferators [Parnell, 1986].

- **oral, rats :**

In a 90-day study described in section V.C.2, a measurement of hepatic microsomal enzyme induction was conducted following exposure to TCA among four groups of male rats ($n=10$) that were administered the compound in their drinking water at concentrations of 0.0, 50.0, 500.0, or 5000.0 ppm. After the 90-day exposure period, livers from the animals were washed in saline, the microsomal fraction was isolated, and total cytochrome-P-450 was determined. Microsomal cytochrome-P-450-dependent O-dealkylation of 7-ethoxycoumarin was assessed and aminopyrine N-demethylase activity was measured.

No dose-dependent changes in total hepatic P-450 content were induced by TCA exposure. Aminopyrine N-demethylase activity was observed in the high dose group only [Parnell, 1986].

- **oral, rats :**

Hepatic enzyme induction by DCA and TCA was investigated in a 90-day study described in section V.C.2. Male Sprague-Dawley rats were divided into four treatment groups (per test compound), with 10 animals/group, and administered DCA or TCA in their drinking water at concentrations of 0.0, 50.0, 500.0, or 5000.0 for 90 days. Fresh liver samples were collected at the time of sacrifice. A portion of the samples were frozen and homogenized, and the ability of the homogenates to oxidize palmitoyl-CoA was measured directly by the oxidation of [1-¹⁴C]palmitoyl. In addition, the conversion of acid insoluble [¹⁴C]palmitoyl CoA to acid soluble [¹⁴C]acetyl CoA was measured by liquid scintillation spectrometry. Liver samples for microsomal enzyme analysis were immediately washed and total cytochrome P-450 was determined. Microsomal cytochrome P-450-dependent O-dealkylation of 7-ethoxycoumarin and aminopyrine N-demethylase activity were also measured.

The livers of rats treated with DCA and TCA at the highest dose level had significantly ($P \leq 0.05$) elevated peroxisomal β -oxidation activity based on the measurement of [¹⁴C]palmitoyl CoA oxidation. DCA was found to be approximately 3 times more active than TCA in inducing hepatic peroxisomal enzymes. No treatment-related effects were observed for either DCA or TCA concerning the measurements of hepatic microsomal enzymes [Mather *et al.*, 1990].

- **oral, rats :**

An investigation was undertaken to determine and compare the effects of TCA and clofibric acid (CA) on peroxisomal proliferation (PP) and the induction of cytochrome P-452 dependent 12-hydroxylation in rats and mice (described in a separate bullet below). PP was determined by measuring the increase in palmitoyl-CoA (PCO) oxidation and confirmed using electron microscopy. An increase in the 12-hydroxylation of lauric acid and a decrease in aminopyrine-N-demethylation were used to indicate the induction of cytochrome P-452.

Groups of 3 male Fisher-344 rats were administered TCA, by gavage, for 10 consecutive days at concentrations of 50.0, 100.0, or 200.0 mg/kg in corn oil. A control group was gavaged with 0.5 ml of corn oil. Because TCA at concentrations of 200 mg/kg was lethal to all of the animals after 6-8 days, the animals in this group were dosed for 5 days only. A positive control group was fed CA (0.2% or 0.4%) blended in NIH-07 open formula mash for 10 consecutive days. It was estimated that the animals consumed 146.1 ± 27 mg/kg (0.2%) or 232.0 ± 73 mg/kg (0.4%), of the CA-mash-feed blend daily. Another control group received intraperitoneal injections of phenobarbital (PB-100.0 mg/kg) for 3 consecutive days to induce cytochrome P-450.

The test animals were sacrificed 24 hours after receiving the final dose of test materials. Body weights were determined and recorded prior to administration of

test materials and before the animals were sacrificed. Their livers were removed, weighed (the liver weight was divided by the terminal body weight to determine liver enlargement or hepatomegaly), and sectioned for electron microscopy. The remaining portion of the liver was pooled (with respective dose group) and prepared for the biochemical assays summarized below.

Hepatomegaly: Hepatomegaly was measured by an increase in the liver to body weight ratio. TCA had no significant effect on the liver/body weight ratio in rats. However, hepatomegaly was significantly increased in the test animals receiving CA and PB ($P < 0.001$ and $P < 0.05$, respectively). No changes were observed in the untreated control groups.

Cytochrome P-450 levels: Cytochrome P-450 levels were determined from the reduced carbon monoxide difference spectrum. The level of cytochrome P-450 was found to be significantly ($P < 0.001$) increased in the 100.0 and 200.0 mg/kg TCA groups compared to the controls. In addition, PB and 0.2% and 0.4% CA significantly ($P < 0.05$) increased cytochrome P-450 levels.

Aminopyrine-N-Demethylation: Aminopyrine was used to determine if the isozyme of cytochrome P-450 induced was different from those induced by phenobarbital. Exposure to TCA was found to cause a significant ($P < 0.05$) dose-dependent decrease in aminopyrine metabolism indicating a reduction in the phenobarbital type isozyme. The observed decreases in TCA and CA were 54% and 50%, respectively.

Palmitoyl-CoA oxidation: PCO oxidase, the enzymatic marker for PP, was measured by the increased rate of *B*-oxidation of the fatty acid PCO. Significant increases, 3- and 10-fold, in PCO oxidation were observed in the animals treated with TCA ($P < 0.001$) and CA ($P < 0.01$), respectively. No increases were observed in the PB treatment group. Electron microscopy revealed increased numbers of peroxisomes in the TCA and CA treatment groups confirming the biochemical findings.

Lauric Acid Hydroxylation: Lauric acid hydroxylation was used to measure the induction of cytochrome P-452. Cytochrome P-452-dependent lauric acid hydroxylation at the 12-position was observed to increase significantly ($P < 0.01$ (TCA) and $P < 0.05$ (CA)) and in a dose-dependent manner following treatment with TCA and CA. The increases were up to 13-fold for TCA and 10-fold for CA. PB increased 12-hydroxylation of lauric acid by 88%, but this increase was not statistically significant.

The ratio of 12-hydroxylation to 11-hydroxylation of lauric acid was determined as a means of detecting whether the test compounds were acting on one specific isozyme or more than one isozyme. An inverse relationship between 12- and 11-hydroxylation of lauric acid was observed. While there was a dose-dependent increase in 12-hydroxylation, there was also a dose-dependent decrease in 11-hydroxylation; thus, the 12/11 ratio increased directly as the dose was increased.

The author concluded from the results of this study that TCA is a peroxisomal proliferator in rats and is capable of inducing cytochrome P-452 dependent 12-hydroxylation of lauric acid. However, the induction of cytochrome P-452 is not dependent upon peroxisome proliferation [Knuckles, 1989].

- **oral, rats :**

The ability of TCA to initiate peroxisome proliferation was studied in rats and mice (described below in a separate bullet). Groups of male Alderley Park rats (Wistar derived, N=4-5) were gavaged, for 10 consecutive days, with doses of TCA in corn oil at concentrations of 0, 10.0, 20.0, 50.0, 100.0 or 200.0 mg/kg. Control groups received the corn oil vehicle only. The animals were sacrificed 24 hours after receiving their last dose. Their livers were weighed, homogenized, and the catalase activity and cyanide insensitive palmitoyl CoA oxidation (peroxisomal B-oxidation marker) were determined spectrophotometrically.

Administration of TCA in rats resulted in a dose-related increase in hepatic peroxisomal B-oxidation (cyanide insensitive palmitoyl CoA oxidation), with a 6.5-fold increase observed at the 200.0 mg/kg dose. Hepatic peroxisomal B-oxidation was increased significantly ($P < 0.05$) over the controls 50 and 100.0 mg/kg dose levels. Peroxisome volume densities increased concomitantly with B-oxidation activity. This compound had no effect upon hepatic catalase activity. Elcombe reported that this study identified TCA as a peroxisomal proliferator in rats [Elcombe, 1985].

- **oral, rats :**

The induction of peroxisomal proliferation (PP) and incidence of renal tubular cell adenocarcinomas from exposure to TCA was observed in rat, in an investigation to determine the existence of a relationship between PP and organ/species differences in carcinogenicity. An unspecified number of male F344 rats were administered, by gavage, 500.0 mg/kg TCA for 10 consecutive days. The peroxisomal specific cyanide insensitive palmitoyl CoA oxidation (PCO) in the test animals' livers and kidneys was significantly (no P value reported) increased by 3- and 1.8-fold, respectively, over the controls. The authors state that the relationship between PP and the carcinogenic response of this compound is presently unknown. The lack of correlation between PP and the carcinogenic response in the kidney suggests that other factors are critical [Goldsworthy *et al.*, 1986].

- oral, rats :

Male Fisher-344 rats were used in a study to determine if peroxisome proliferation may occur in the kidney as previously described for the liver. A parallel study was conducted on mice (see separate bullet below). For the first experiment, six animals were gavaged daily, for 10 consecutive days, with 500.0 mg/kg TCA dissolved in corn oil. A control group (5 animals) was treated with the corn oil vehicle only. At the conclusion of the study, the animals were sacrificed and weighed. The liver and kidney weights were recorded and samples from each organ, left lobe of the liver and the kidney cortex were obtained for enzyme analysis. In addition, the palmitoyl CoA oxidase (PCO) activity, an indicator of peroxisomal *B*-oxidation activity, was measured.

Results obtained indicate that TCA has little or no effect on body weight gain. The animals liver to body weight ratios were significantly increased ($P < 0.05$) over the controls. Likewise, significant increases in the hepatic cyanide-insensitive PCO were observed ($P < 0.05$) compared to controls. The kidney to body weights and renal PCO activity was not significantly impacted by exposure to this compound. The authors state that the role of TCA concerning peroxisome proliferation and tumor induction in rats remains to be established.

A second experiment was conducted to determine whether the vehicle (corn oil) used had any influence on PCO activity. Six male Fisher-344 rats were gavaged with 500.0 mg/kg TCA dissolved in either corn oil or methyl cellulose for 10 consecutive days. Naive animals received no treatment and vehicle controls received the vehicle alone. The results of this study supported the findings of the first experiment and confirmed that PCO activity is not influenced by either corn oil or methyl cellulose [Goldsworthy and Popp, 1987].

- oral, rats :

The effect of different test vehicles on TCA's ability to induce peroxisome proliferation was also studied by DeAngelo *et al.* (1989). A group of male Fisher 344 rats were given, via gavage, 200.0 mg/kg TCA dissolved in either corn oil or distilled water for 10 days. Twenty four hours after the last dose was administered, the animals were sacrificed and the influence of TCA in corn oil and water on palmitoyl CoA oxidase (PCO) activity was compared.

PCO activity was significantly ($P < 0.05$) increased compared to the control vehicle by TCA in both vehicles. Corn oil and TCA independently increased PCO activity to 140% and 179% of the water control, respectively; however, TCA dissolved in corn oil increased PCO activity 228% of the value obtained for the corn oil control and 314% of the water control. The authors state that the vehicle used is an important consideration. In this study, corn oil alone was observed to increase PCO activity thus it can potentially enhance the PCO activity, of TCA [DeAngelo *et al.*, 1989].

oral rats :

The induction of peroxisome proliferation by DCA and TCA was studied in several strains of rats by measuring the cyanide-insensitive palmitoyl Coenzyme A (PCO) and carnitine acetyl-CoA transferase (CAT) activities. Male Sprague-Dawley (S-D) rats were exposed to DCA at concentrations of 8.0, 16.0, and 39.0 mM and TCA at concentrations of 6.0, 21.0, and 31.0 mM. In addition, an identical study was run concurrently on groups of 6 Osborne-Mendel (O-M) and Fisher 344 (F-344) rats exposed to 12.0 and 31.0 mM TCA, only in their drinking water for 14 days. A control group received sodium chloride in their drinking water. At the conclusion of the study, the animals were sacrificed. Liver and body weights were recorded and expressed as a percentage of body weight. The liver was removed and prepared to evaluate the induction of peroxisome proliferation and CAT activity. The enhancement of peroxisome proliferation (at the highest test concentration only) was scored by determining the number of peroxisomes per unit volume of cytoplasm and calculating the percentage of the cytoplasm they occupied.

At the conclusion of this study, it was determined that a dose-dependent depression in weight gain was observed in the S-D rats. Relative liver weights were not affected by either compound. TCA caused no significant changes in body weight of either O-M or F-344 rats, although the weight of the F-344 rats was decreased slightly. TCA administered at a dose of 31 mM significantly increased ($P<0.05$) liver weight in F-344 rats.

DCA significantly ($P<0.05$) enhanced CAT activity in S-D rats in the medium and high dose groups. In the high dose group (39 mM), liver CAT activity was increased 1033% over the control. TCA had a significant ($P<0.05$) increase in CAT only in the 31 mM dose group (321% of control). PCO activity in S-D rats was not increased by either DCA or TCA, except in the 39 mM DCA dose group. TCA increased PCO activity significantly ($P<0.05$) in the 31 mM dose group of F-344 and O-M rats by 163% and 238% of the control value, respectively. No increase in the number of peroxisomes was observed and the volume of the cytoplasm occupied was not significantly increased.

DCA had a stronger effect on hepatic CAT activity than TCA. TCA was a relatively weak inducer of peroxisomes in the O-M and F-344, but not in S-D rats [DeAngelo *et al.*, 1989].

- ***oral, mice :***

In conjunction with the study described above, the ability of DCA and TCA to stimulate peroxisomal proliferation in several strains of mice was tested using the same experimental design. B6C3F1 mice received DCA and TCA in drinking water at concentrations of at 8, 16, and 39 mM and 6, 21, and 31 mM, respectively, for 14 days. In addition, groups of Swiss-Webster, C57BL/6 and C3H mice received doses of 12 and 31 mM TCA in their drinking water to compare the effects of TCA in different strains of mice. Control group were given sodium chloride (NaCl) in their drinking water.

At the time of sacrifice, it was found that neither DCA nor TCA affected weight gain in any of the mouse strains. The relative liver weights in the B6C3F1 mice were significantly ($P<0.01$) increased by DCA (in all dose groups) and TCA (31 mM only). Both doses (12 and 31 mM) of TCA significantly ($P<0.05$) increased relative liver weights in the Swiss-Webster, B6C3F1, C57BL/6, and C3H mice.

DCA and TCA significantly ($P<0.05$) enhanced CAT activity in B6C3F1 mice at a dose of 31 mM. TCA (31 mM) increased liver CAT activity 561% over the control. PCO activity in B6C3F1 mice was increased significantly ($P<0.05$) by both DCA (31 mM) and TCA (12 and 31 mM). TCA increased PCO activity significantly ($P<0.05$) over the controls in the 12 and 31 mM dose groups of Swiss-Webster (376% and 748%), B6C3F1 (556% and 778%), C57BL/6 (2592% and 2213%), and C3H mice (425% and 744%).

The number of peroxisomes from DCA and TCA treated B6C3F1 mice were observed to be significantly ($P<0.05$) increased (30.77 ± 2.85 and 30.75 ± 2.80 , respectively), compared to the control (6.89 ± 1.23). The volume percentage of the cytoplasm occupied by the peroxisomes was also significantly ($P<0.05$) increased by DCA and TCA over the control ($3.75\pm 0.46\%$, $4.92\pm 0.54\%$, and $0.61\pm 0.12\%$, respectively).

The authors concluded that TCA is a more potent peroxisome proliferator than DCA in all strains of mice tested. In addition, TCA and DCA were more effective enhancers of hepatic peroxisome proliferation in the mice than in the rat (previous bullet). The role played by peroxisome proliferation in the carcinogenicity of DCA and TCA in mouse liver could not be determined from the results of this study [DeAngelo *et al.*, 1989].

- oral mice :

An investigation was carried out to determine and compare the effects of TCA and clofibrac acid (CA) on peroxisomal proliferation (PP) and the induction of cytochrome P-452 dependent 12-hydroxylation of lauric acid in mice. This study was also conducted in rats as described previously using the same experimental design (see Knuckles, 1989).

Groups of 3 male B6C3F1 mice were administered TCA, by gavage, for 10 consecutive days at concentrations of 50.0, 100.0, or 200.0 mg/kg in 0.25 ml corn oil. A control group was gavaged with 0.25 ml of corn oil only. All of the mice dosed with 200.0 mg/kg TCA died within 3 days. To determine whether the mice died as a result of the intrinsic toxicity of TCA or because of the high acidity of the compound, a dose of 200.0 mg/kg was dissolved in 50.0 mm tris-HCl buffer (ph 7.5). Mice were dosed with this test solution for 5 consecutive days; controls were treated with tris-HCl only. A positive control group were fed CA (0.2% or 0.4%) blended in NIH-07 open formula mash for 10 consecutive days. It was estimated that the animals consumed 322.8 ± 44.5 mg/kg (0.2%) or 649.5 ± 149 mg/kg (0.4%) of the CA-mash feed blend, daily. Another control group (positive for the induction of cytochrome (P-450) received intraperitoneal injections of phenobarbital (PB-100 mg/kg) for 3 consecutive days. The test animals were sacrificed 24 hours after receiving the final dose of test materials. Body weights were determined and recorded prior to administration of test materials and sacrifice. The liver was removed, weighed, and sliced for electron microscopy. The remaining portion of the liver was pooled (by respective dose group) and prepared for biochemical evaluation.

Hepatomegaly: TCA, CA, and PB significantly increased ($P < 0.001$) hepatomegaly. The liver/body weight ratio in mice was increased by 20 and 23% for the 50.0 and 100.0 mg/kg TCA dose group and by 8% and 24% for 0.2% and 0.4% CA groups, and by 27% for the PB group, respectively. There was no significant increase in hepatomegaly of the animals receiving 200.0 mg/kg TCA. However, the 200.0 mg/kg dose group did appear to have suffered some adverse effects including: ruffled fur and distended intestines, and the appearance of their livers was grey with some necrotic spots. The test animals that were given 200.0 mg/kg TCA in tris buffer, were observed to have significantly ($P < 0.001$) increased liver weights and hepatomegaly, but none of the other effects previously described. No changes were observed in the untreated control groups.

Cytochrome P-450 levels: The level of cytochrome P-450 was significantly ($P < 0.001$) increased over the controls by all of the test compounds. TCA increased the level of cytochrome P-450 by 72%. PB and 0.2% and 0.4% CA increased cytochrome P-450 by 2.5-fold and 62%, respectively, compared to control levels. PB significantly ($P < 0.05$) increased the N-demethylation of aminopyrine to yield formaldehyde. A dose-dependent decrease in aminopyrine metabolism was observed. Aminopyrine-N-demethylation was decreased by approximately 80% and 50% by TCA and CA, respectively.

Aminopyrine-N-Demethylation: For clofibric acid and TCA, a significant ($P<0.05$) dose-dependent decrease in aminopyrine metabolism was observed, indicating a reduction in the phenobarbital-type isozyme. The reduction in aminopyrine-N-demethylation was decreased by approximately 50% and 80% for clofibric acid and TCA respectively, compared to controls.

Palmitoyl-CoA oxidation: Significant increases in the oxidation rate of PCO were observed. Rate increases of 5.4- and 6-fold in the animals treated with TCA ($P<0.001$ and $P<0.05$ (TCA in tris)) and CA ($P<0.05$), respectively were noted. No increases were observed in the PB treatment group indicating the absence of PP. Electron microscopy confirmed the biochemical findings of increased numbers of peroxisomes in the TCA and CA treatment groups.

Lauric Acid Hydroxylation: Cytochrome P-452 dependent lauric acid hydroxylation at the 12-position was observed to increase significantly and in a dose-dependent manner for all of the treatments. The increases were up to 15-fold for TCA, 17-fold for CA, and 10-fold for PB compared to control levels. The ratios of 12-hydroxylation to 11-hydroxylation of lauric acid were increased in a dose-dependent manner. The 12/11 ratio for TCA ranged from 1.5-2.5, indicating greater induction of 12-hydroxylation. The 12/11 ratio for CA approached 1, indicating roughly equal induction of isozymes responsible for the 11- and 12-lauric acid hydroxylation. For PB samples, the isozyme responsible for 12-hydroxylation of lauric acid also appeared to be induced to a greater extent.

The author concluded from the results of this study that TCA is a peroxisomal proliferator in mice and is capable of inducing cytochrome P-452 dependent 12-hydroxylation of lauric acid. However, the induction of cytochrome P-452 is not dependent upon peroxisome proliferation [Knuckles, 1989].

- **oral, mice:**

TCA's ability to initiate peroxisome proliferation was studied in mice and rats (described previously (Elcombe, 1985)). Groups of male Alderley Park mice (Swiss derived, $N=4-5$) were gavaged, for 10 consecutive days, with doses of TCA in corn oil at concentrations of 0, 10.0, 20.0, 50.0, 100.0, and 200.0 mg/kg. Control groups received corn oil only. The animals were sacrificed 24 hours after receiving their last dose. Their livers were weighed, homogenized, and the catalase activity and cyanide insensitive palmitoyl CoA oxidation (peroxisomal *B*-oxidation marker) were determined spectrophotometrically.

Administration of TCA in mice resulted in a dose-related increase in hepatic peroxisomal *B*-oxidation (cyanide insensitive palmitoyl CoA oxidation). A 4.8-fold increase was observed at the 200 mg/kg dose. Hepatic peroxisomal *B*-oxidation was increased significantly ($P<0.05$) over the controls at doses of 100.0 and 200.0 mg/kg only. Peroxisome volume densities increased as expected with increased *B*-oxidation activity. TCA had no effect upon hepatic catalase activity. TCA was concluded to be a peroxisomal proliferator in mice [Elcombe, 1985].

- **oral, mice :**

Male B6C3F1 mice were used in a study to determine if peroxisome proliferation induced by TCA may occur in the kidney as previously described for the liver. A parallel investigation was carried out with rats (see Goldsworthy and Popp, 1987). Eight animals were gavaged daily, for 10 consecutive days, with TCA dissolved in corn oil at a dose of 500 mg/kg body weight. A control group (7 animals) was treated with the corn oil vehicle only. At the conclusion of the study, the animals were sacrificed and weighed. The liver and kidney weights were recorded and samples from each organ, the left lobe of the liver and the kidney cortex, were obtained for enzyme analysis. Palmitoyl CoA oxidase (PCO) activity, an indicator of peroxisomal *B*-oxidation activity, was determined.

Results obtained indicate that TCA has little or no effect on body weight gain. The animals liver to body weight ratios were significantly increased ($P < 0.05$) over the controls. Likewise, significant increases in the hepatic cyanide-insensitive PCO were observed ($P < 0.05$). The kidney to body weight ratios were not significantly increased, however the renal PCO activity was significantly increased ($P < 0.05$) by this compound. The authors state that the role of TCA with respect to peroxisome proliferation and tumor induction in mice remains to be established [Goldsworthy and Popp, 1987].

- **in vitro, rats :**

A study was conducted to investigate the mechanism of TCA-induced peroxisomal proliferation and its relationship to hepatocarcinogenesis. Hepatocytes were isolated and cultured from male Alderley Park rats (Wistar derived). TCA at concentrations of 0.01-5.0 mM was dissolved in dimethylformamide (DMF) and added to the hepatocyte cultures every 24 hours, for 72 hours, after which the medium was changed. Peroxisomal *B*-oxidation was measured in the cell homogenates as palmitoyl-CoA (PCoA) dependent reduction of NAD^+ in the presence of CN^- to inhibit the mitochondrial system.

TCA was found to induce a time- and dose-dependent increase in peroxisomal enzyme activity. The greatest amount of PCoA activity was induced by 5.0 mM TCA. The authors state that the investigation of the phenomenon of hepatic peroxisome proliferation and its postulated role in hepatocarcinogenesis is complicated by three important factors: 1) the role of the peroxisome in normal cell metabolism is unclear, 2) the mechanism(s) involved in hepatic peroxisome proliferation is unknown, as is the contributor, if any, of this altered metabolic status to hepatocarcinogenesis, and 3) interspecies differences in response to peroxisome proliferators makes extrapolation of this data to the prediction of human effects extremely difficult [Mitchell *et al.*, 1984].

- *in vitro, rats:*

The effects of TCA on hepatocyte intracellular communication have been studied by Klaunig *et al.* (1989). Hepatocytes were isolated from male Fischer 344 rats and male B6C3F1/CrlBr mice. The gap junction mediated intercellular communication was assessed by injecting fluorescent dye into one hepatocyte, the "donor" and observing it "spread" to an adjacent cell. Twenty-four-hour-old primary hepatocyte cultures and freshly plated cells were exposed to doses of TCA at concentrations of 1×10^{-4} , 5×10^{-4} , and 1×10^{-3} M. Positive controls were dosed with phenobarbital (PB) a known intercellular communication inhibitor. The dye coupling in the 24 hour old cultures was assessed 4, 8, and 24 hours after treatment and after 3 and 6 hours of treatment in the fresh cultures.

TCA had no influence on either the fresh or the 24-hour-old rat hepatocyte cultures. In the 24-hour-old mouse cultures, transient inhibition of dye coupling was observed after 4 hours. PB inhibited dye coupling in the 24-hour old culture after 4, 8 and 24 hours. In the fresh cultures, TCA had no effect on rat cultures but significantly ($P < 0.05$) inhibited intercellular communication in the mouse hepatocytes after 3 and 6 hours, with greater inhibition seen after 6 hours. PB was inhibitive after 3 and 6 hours in a dose-dependent manner.

The authors stated that because TCA failed to inhibit intercellular communication in rats, and induced peroxisomal proliferation [Elcombe, 1985] these two actions have different processes. Because no intercellular communication inhibition was observed in rat hepatocytes, the authors suggest a preferential susceptibility of mouse hepatocytes to TCA may exist. The authors stated that the results suggest that the peroxisome proliferation process may not be related to the inhibition of cellular communication because TCA induces peroxisome proliferation in both rat and mouse hepatocytes [Elcombe, 1985], but failed to inhibit intracellular communication in rat hepatocytes. They continued that the preferential susceptibility of mouse hepatocytes observed in this study suggests that mouse hepatocytes may possess other intrinsic properties that make them more sensitive to TCA which may be the reason for their increased hepatocarcinogenic sensitivity [Klaunig *et al.*, 1989].

- *in vitro, human/rat/mouse:*

The ability of TCA to cause peroxisome proliferation in human, rat, and mouse hepatocytes was studied in an *in vitro* investigation. Hepatocytes were isolated from their respective liver donors and incubated with TCA (0-5 mM) dissolved in N, N-dimethylformamide for 3 days. Ninety-six hours after seeding, the hepatocyte cells were harvested and the extent of cyanide insensitive palmitoyl CoA oxidation was determined. Dose-related increases in cyanide insensitive palmitoyl CoA oxidation were observed in the mouse and rat hepatocytes, but not in the human hepatocytes. The authors stated that, based on the results, TCA is not a peroxisome proliferator in humans [Elcome, 1985].

Single Strand Break Induction

- oral, rats:

The ability of DCA and TCA to induce single-strand breaks (SSBs) in the hepatic DNA of rats was evaluated using an alkaline unwinding assay, which measures the rate of transition of double-stranded DNA to single-stranded DNA during alkaline denaturation (This test was also conducted in mice, as described below.) Groups of 4 male Sprague-Dawley rats were administered a single oral dose of 80.0 mg/kg DCA or TCA in a 1% aqueous solution of Tween (total volume of 1 ml). Control animals received equivalent amounts of the vehicle. The animals were sacrificed 4 hours after treatment, liver fractions were isolated and prepared, and the number of SSBs in the DNA was determined⁶.

The results of the study show that DCA is a more potent inducer of SSB than TCA. The lowest concentrations of DCA and TCA needed to significantly increase ($P < 0.05$) the rate of alkaline unwinding were 0.23 mmol/kg and 0.6 mmol/kg, respectively. The dose-response curves obtained from rats were uniformly steep with slopes (and 95% confidence intervals) of 0.29 ± 0.02 and 0.31 ± 0.06 , respectively. When results were compared to the results obtained in a parallel study using trichloroethylene (TCE), it was found that higher doses of TCE were needed to induce an equivalent number of SSBs. The author concluded that, in this case, SSBs resulting from TCE administration were due to one or more of TCE's metabolites [Nelson, 1989].

- oral, rats:

The ability of DCA and TCA to cause DNA strand breaks in rat liver *in vivo* was studied in male F-344 rats. An unspecified number of animals were given a single oral dose of TCA or DCA at 1.0, 5.0, or 10.0 mmol/kg. Positive control groups were given 1 mmol/kg diethylnitrosamine or 0.5 mmol/kg methyl methanesulfonate (MMS). Four hours after treatment, the number of strand breaks in liver cells and splenocytes were evaluated using an alkaline unwinding assay. No significant increases in strand breaks from exposure to either DCA or TCA were observed in the liver cells or splenocytes; however, a significant increase (P value not reported) did occur in liver cells from both positive controls and in the splenocytes following exposure to MMS. [Chang *et al.*, 1989].

⁶ The fraction of DNA unwound was calculated using the following equation: (The subscripts indicate the amount of double stranded DNA at time 0 and "t" refers to the incubation time in alkaline solution.)

$$\frac{(\text{Total DNA} - \text{double stranded DNA})_t}{(\text{Total DNA})} - \frac{(\text{Total DNA} - \text{double stranded DNA})_0}{(\text{Total DNA})}$$

- **oral, mice:**

The procedure described in the previous bullet was repeated using male B6C3F1 mice. Again, it was concluded that neither DCA nor TCA cause DNA strand breaks in liver cells and splenocytes after short term exposure [Chang *et al.*, 1989].

- **oral, mice:**

The ability of DCA and TCA to induce single-strand breaks (SSBs) in the hepatic DNA of mice was evaluated using an alkaline unwinding assay, which measures the rate of transition of double-stranded DNA to single-stranded DNA during alkaline denaturation (this test was also conducted in rats, as described above). Groups of 4 male B6C3F1 mice were administered a single oral dose of 80.0 mg/kg DCA or TCA in a 1% aqueous solution of Tween 80 (total volume of 1 ml). Control animals received equivalent amounts of the vehicle. The animals were sacrificed 4 hours after treatment, liver fractions were isolated and prepared, and the number of SSBs in the DNA was determined.

As seen in the rat study, a dose-dependent increase in SSBs was observed following exposure to DCA and TCA. However, the results of this study show that TCA is a more potent inducer of SSB than DCA. A 0.006 mmol/kg dose of TCA was required to produce a significant increase ($P < 0.05$) in the rate of alkaline unwinding, while the same dose of DCA produced SSBs, but a smaller magnitude. The dose-response curves obtained from mice were not as steep as those calculated in rats. DCA had a slope of 0.11 ± 0.02 , and TCA had a slope of 0.13 ± 0.01 . The author concluded that the dose-response relationships observed suggest differences in the mechanisms by which strand breaks are induced in the hepatic DNA of the rat and mouse [Nelson, 1989].

- **oral, mice:**

Four experiments were conducted to determine whether the ability of DCA and TCA to induce single strand breaks (SSBs) is dependent upon peroxisomal proliferation (PP). Male B6C3F1 mice were used in each of the experiments.

Experiment 1: The alkaline unwinding assay was used to measure the number of SSBs in DNA between 1 and 24 hours. Three groups of 30 mice were gavaged with a single dose of DCA (0.08 or 3.9 mmol/kg) or TCA (3.1 mmol/kg) dissolved in 1% aqueous Tween 80. The control group was dosed with an equivalent amount of vehicle. The animals (N=6 animals from each test group/time point) were sacrificed 1, 2, 4, 8, or 24 hours after administration of the test materials. The fraction of DNA unwound was calculated using the equation described in footnote #6.

Experiment 2: PP was determined by measuring palmitoyl-CoA oxidation (PCO) in liver homogenates. Four groups of 30 mice were administered a single dose of DCA (0.08 or 3.9 mmol/kg), TCA (3.1 mmol/kg), or the control vehicle, by gavage. The animals (N=6 animals from each test group/time point) were sacrificed 1, 2, 4, 8, and 24 hours after administration of the test materials and liver homogenates were prepared to measure PCO.

Experiment 3: Groups of 6 animals were administered, by gavage, either DCA (3.9 mmol/kg), TCA (3.1 mmol/kg), or control vehicle for 10 consecutive days. A positive control group given clofibrate at 250 mg/kg/day was included. Twenty-four hours after administration of the final dose of test compound, the animals were sacrificed and their livers extracted, weighed and prepared for microscopic examination and determination of PCO.

Experiment 4: The number of SSBs was measured in hepatic DNA in 5 mice administered 3.1 mmol/kg TCA by gavage, for ten consecutive days. A control group of 5 mice (N=5) were administered vehicle only. The animals were sacrificed 24 hours after the final dose of test material was given.

Both DCA and TCA significantly ($P \leq 0.05$) increased the rate of alkaline unwinding 1, 2, and 4 hours after administration of test materials. The greatest number of breaks were observed after 1 hour. After 8 hours, the rate of unwinding was within the range observed by the control group. However, SSBs in animals repeatedly administered TCA were not significantly different from the controls.

Mice treated with single doses of DCA and TCA showed no increase in PCO activity (PP was not induced). Repeat dosing of DCA and TCA had no effect upon body weight gain in the test animals compared to the control group. However, both compounds significantly ($P < 0.05$) increased liver and liver to body weight ratios. Weight changes induced by DCA were significantly ($P < 0.05$) greater than those affected by TCA. Repeated dosing with TCA and DCA also increased PCO activity significantly ($P < 0.05$) compared to the control group. TCA-induced PCO activity was significantly ($P < 0.05$) higher than that induced by DCA. PCO activity after treatment with clofibrate (positive control) was significantly ($P < 0.05$) increased over the TCA treatment group.

Histopathology of the test animals' livers showed that peroxisome profile counts were significantly higher in the DCA and TCA treated animals than in the controls with no discernible differences between the two treatment groups. Peroxisomes in the DCA treated livers generally lacked the pronounced nucleoid core observed in the TCA and control groups. No evidence of gross hepatotoxicity was seen in the control or TCA treatment groups. Hematoxylin and eosin stained liver sections of the TCA and control groups showed no abnormality. Hepatocytes of the animals repeatedly treated with DCA were remarkably larger (1.4 times) than the control. Marked cellular hypertrophy and necrosis were observed throughout the liver.

The author concluded from the results of the 4 experiments that peroxisomal proliferation does not contribute to the induction of SSBs in hepatic DNA of mice [Nelson, 1989].

VI. STRUCTURE ACTIVITY RELATIONSHIPS

The carcinogenicity of dichloroacetate and trichloroacetate have been investigated in B6C3F1 male and female mice. Mice were administered dichloroacetate and trichloroacetate in their drinking water at concentrations of 1.0 or 2.0 g/L for up to 52 weeks. Both compounds were found to induce hepatoproliferative lesions (HPL) in male mice which included hepatocellular nodules, adenomas, and hepatocellular carcinomas within 12 months. The induction of HPL by trichloroacetate was found to be linear with dose. However, the response to dichloroacetate increased sharply with the increase in concentration from 1.0 to 2.0 g/L. Suspension of dichloroacetate treatment at 37 weeks was found to result in the same number of HPL at 52 weeks that would have been predicted on the basis of the total dose administered. However, none of the lesions progressed to hepatocellular carcinomas when treatment was stopped at 37 weeks. On the other hand, suspension of trichloroacetate treatment at 37 weeks caused a decrease in HPL compared to the number of HPL observed at 52 weeks. Throughout treatment, dichloroacetate-treated mice had enlarged livers characterized by a marked cytomegaly and massive accumulations of glycogen in hepatocytes and areas of focal necrosis were observed throughout the liver. Lipofuscin accumulation was less well marked than that observed for trichloroacetate. Trichloroacetate induced small increases in cell size and a smaller amount of glycogen. Focal necrotic damage did not occur in trichloroacetate-treated animals. Trichloroacetate produced marked accumulations of lipofuscin in the liver. The authors report that these data indicate that dichloroacetate and trichloroacetate are capable of inducing hepatic tumors in B6C3F1 mice and conclude that the mechanisms involved in tumor induction differ substantially between the two compounds. They state that tumorigenesis by dichloroacetate may depend largely on stimulation of cell division secondary to hepatic damage. On the other hand, trichloroacetate appears to increase lipid peroxidation, suggesting that production of radicals may be responsible for its effects [Bull *et al.*, 1990].

No other data were found on the carcinogenicity of dichloroacetate and trichloroacetate [Cancerlit, 1991].

Carcinogenicity studies were conducted by administering monochloroacetic acid in deionized water to groups of F344/N rats and B6C3F1 mice of each sex, once daily for 2 years. The test concentrations used were 0.0, 15.0, or 30.0 mg/kg and 0.0, 50.0, or 100.0 mg/kg for rats (N = 70/group) and mice (N = 60/group), respectively. Under the conditions of the study, there was no evidence of carcinogenic activity for male or female rats or mice [National Toxicology Program, 1990 (draft report)].

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APPENDIX I. ON-LINE DATABASES SEARCHED

DATE OF SEARCH

BRS:

HZDB May, 1991

DIALOG:

Agricola May, 1991
Agris International May, 1991
Aquatic Sciences Abstracts May, 1991
Biosis Previews May, 1991
CAB Abstracts May, 1991
Cancerlit May, 1991
Chem Bus Newsbase May, 1991
Chemical Exposure May, 1991
Compendex Plus May, 1991
CRIS USDA May, 1991
Embase May, 1991
Enviroline May, 1991
Environmental Bibliography May, 1991
Federal Register May, 1991
Foods Adlibra May, 1991
FSTA May, 1991
Life Sciences Collection May, 1991
Medline May, 1991
NTIS May, 1991
Occu. Safety and Health May, 1991
PTS Newsletter May, 1991
PTS Prompt May, 1991
Pollution Abstracts May, 1991
Trade and Industry ASAP May, 1991

MEAD:

Nexis/Lexis-BNA ENV May, 1991

NLM:

Chemline May, 1991
HSDB May, 1991
RTECS May, 1991
Toxline 65 May, 1991
Toxline May, 1991
Toxlit May, 1991
Toxlit 65 May, 1991

STN:

CA May, 1991
Chemlist May, 1991

APPENDIX II. SAFETY INFORMATION

• HANDLING AND STORAGE

DCA and TCA are classified as corrosive [Aldrich, 1990; Sax and Lewis, 1989]. DCA is stable under normal laboratory conditions. This compound reacts with water or steam to produce toxic and corrosive fumes [Sax and Lewis, 1989]. It is incompatible with strong oxidizing agents, strong bases, and strong reducing agents. Decomposition of DCA produces toxic fumes of carbon monoxide, carbon dioxide, and hydrogen chloride gas [Lenga, 1988].

TCA should be stored in a tightly closed container in a cool place. Solutions of TCA shall be stored in water of less than 30% strength. TCA is incompatible with strong oxidizing agents and strong bases [Lenga, 1988]. When heated to decomposition, it emits toxic fumes of Cl- and Na₂O [Sax and Lewis, 1980]. Other decomposition products include chloroform, alkali carbonate [Budavari, 1989], hydrochloric acid [Budavari, 1989], carbon dioxide, carbon monoxide [Budavari, 1989; Lenga, 1988], and hydrogen chloride gas [Lenga, 1988].

• EMERGENCY FIRST AID PROCEDURES

Eye: First check the victim for contact lenses and remove if present. Flush victim's eyes with water or normal saline solution for 20 to 30 minutes while simultaneously calling a hospital or poison control center. Do not put any ointments, oils, or medication in the victim's eyes without specific instructions from a physician. Immediately transport the victim to a hospital even if no symptoms (such as redness or irritation) develop.

Skin: IMMEDIATELY flood affected skin with water while removing and isolating all contaminated clothing. Gently wash affected skin areas thoroughly with soap and water. If symptoms such as inflammation or irritation develop, IMMEDIATELY call a physician or go to a hospital for treatment.

Inhalation: IMMEDIATELY leave the contaminated area and take deep breaths of fresh air. If symptoms (such as wheezing, coughing, shortness of breath, or burning in the mouth, throat, or chest) develop, call a physician and be prepared to transport the victim to a hospital.

Provide proper respiratory protection to rescuers entering an unknown atmosphere. Whenever possible, Self-Contained Breathing Apparatus (SCBA) should be used.

Ingestion: DO NOT INDUCE VOMITING. Corrosive chemicals will destroy the membranes of the mouth, throat, and esophagus and, in addition, have a high risk of being aspirated into the victim's lungs during vomiting which increases the medical problems.

If the victim is conscious and not convulsing, give 1 or 2 glasses of water to dilute the chemical and IMMEDIATELY call a hospital or poison control center. IMMEDIATELY transport the victim to a hospital.

If the victim is convulsing or unconscious, do not give anything by mouth, ensure that the victim's airway is open and lay the victim on his/her side with the head lower than the body. DO NOT INDUCE VOMITING. Transport the victim IMMEDIATELY to a hospital.

- **PROTECTIVE EQUIPMENT**

Eye: Safety goggles

Gloves: Two pairs of dissimilar protective gloves shall be worn when handling the neat chemical, otherwise one pair. When contact with this chemical has been known to occur, change gloves immediately.

Clothing: Minimally, a disposable laboratory suit (e.g. Tyvek ®) shall be worn, as specified in the most current NTP Statement of Work or the NTP Health and Safety Minimum Requirements.

Respiratory

Protection: A NIOSH-approved chemical cartridge respirator with an organic vapor and high-efficiency particulate filter cartridge.

- **EXTINGUISHANT**

Dry chemical, carbon dioxide or halon extinguisher.

- **MONITORING PROCEDURES**

There is no NIOSH analytical method reported in the NIOSH Manual of Analytical Methods for DCA or TCA.

- **SPIILLS AND LEAKAGE**

Persons not wearing the appropriate protective equipment and clothing shall be restricted from areas of spills until cleanup has been completed. When exposure to unknown concentrations may occur, air-purifying respirators may not be used. Chemical cartridge respirators with organic vapor cartridges may not be used when airborne concentrations exceed 1000 ppm.

If DCA or TCA are spilled the following steps shall be taken:

1. In order to prevent dust formation, use moistened paper towels to clean up a solid spill. Avoid dry sweeping.

2. If a liquid solution is spilled, use vermiculite, sodium bicarbonate, sand, or paper towels to contain and absorb the spill.
 3. Clean the spill area with dilute alcohol (approximately 60-70%) followed by a strong soap and warm water washing.
 4. Dispose of all absorbed material as hazardous waste.
- **DECONTAMINATION OF LABORATORY EQUIPMENT**

TDMS Terminal: Whenever feasible, a protective covering (e.g., plastic wrap) shall be placed over the keyboard when in use.

General Equipment: Before removing general laboratory equipment (i.e., lab carts, portable hoods and balances) from animal dosing rooms and/or chemical preparation areas, a decontamination process shall be conducted in addition to routine housekeeping procedures.

- **WASTE MANAGEMENT AND DISPOSAL PROCEDURES**

Waste Management: If an inhalation study is to be conducted, all exhaust air from the inhalation chamber must be cleaned with appropriate air cleaning devices unless the laboratory has informed local and state air pollution regulatory agencies of both the laboratory's operating practices and the potential hazards of the chemical's in use. Compliance with all federal, state and local air pollution laws and regulations is required. A specific air cleaning system design must consider the specific conditions of the laboratory (eg., air flow rates and volumes, mixing of exhaust streams, size of inhalation chamber, etc.) and the dosing regimen selected. Air cleaning systems designs must be described by the laboratory and approved by the NTP Office of Laboratory Health and Safety.

Waste Disposal: Securely package and label, in double bags, all waste material. All potentially contaminated material (i.e., carcasses, bedding, disposable cages, labware) shall be disposed of by incineration in a manner consistent with federal (EPA), state, and local regulations or disposed of in a licensed hazardous waste landfill.