201-15628

*Safety, Health & Environment Excellence Center*
*1007 Market Street, DuPont 6082*
*Wilmington, DE 19898*
*302-773-0910 (Office) – 302-774-3140 (Fax)*

September 29, 2004

RECEIVED
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Marianne Lamont Horinko, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 2216

Attn: Chemical Right-to-Know Program

Dear Administrator Horinko,

E. I. du Pont de Nemours & Company, Inc. is please to submit the proposed test plan along with the robust summary for the chemical, dichloroacetyl chloride, CAS# 79-36-7. DuPont understands there will be a 120-day review period for the test plan and that all comments received by the EPA will be forwarded to DuPont for consideration.

This submission includes one electronic copy in .pdf format.

Please contact me by phone at (302) 773-0910 or by e-mail, Edwin.L.Mongan-1@usa.dupont.com, if you have any questions regarding this matter.

Sincerely,

Edwin L. Mongan, III
Manager, Environmental Stewardship
DuPont Safety, Health & Environment

Cc: Charles Auer – U.S. EPA
Office of Pollution Prevention & Toxics
U. S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

## OVERALL SUMMARY FOR DICHLOROACETYL CHLORIDE

For the purposes of this document, dichloroacetyl chloride (also known as DCAC) data were searched and summarized. Data were also identified for the hydrolysis product, dichloroacetic acid (DCA). The available data for these chemicals is presented in Table 1. Each study on these materials was evaluated for adequacy. Robust summaries were developed for each study addressing specific SIDS endpoints. Summaries were also developed for studies either considered not adequate but that provided information of relevance for hazard identification and evaluation, or covered non-SIDS endpoints (Appendices A and B).

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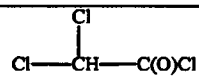
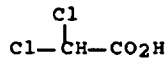
**Table 1: Matrix of Available and Adequate Data**

| | Dichloroacetyl chloride (DCAC) | Dichloroacetic acid (DCA) |
|---|---|---|
| **CAS Registry No.** | 79-36-7 | 79-43-6 |
| **STRUCTURE** |  |  |
| **PHYSICAL/CHEMICAL CHARACTERISTICS** | | |
| Melting Point | N/A | √ |
| Boiling Point | √ | √ |
| Density | √ | √ |
| Vapor Pressure | √ | √ |
| Partition Coefficient | √/- | √ |
| Water Solubility | √ | √ |
| **ENVIRONMENTAL FATE** | | |
| Photodegradation | √ | √ |
| Stability in Water | √ | √ |
| Transport (Fugacity) | √ | √ |
| Biodegradation | √ | √ |
| **ECOTOXICITY** | | |
| Acute Toxicity to Fish | √* | √ |
| Acute Toxicity to Invertebrates | √* | √ |
| Acute Toxicity to Aquatic Plants | √* | √ |
| **MAMMALIAN TOXICITY** | | |
| Acute Toxicity | √ | √ |
| Repeated Dose Toxicity | √* | √ |
| Developmental Toxicity | √* | √ |
| Reproductive Toxicity | √* | √ |
| **GENETIC TOXICITY** | | |
| Genetic Toxicity Gene Mutations | √ | √ |
| Genetic Toxicity Chromosomal Aberrations | √* | √ |

√ = Data are available and considered adequate.
√/– = Data are available, but considered inadequate.
– = No data available.
N/A = Not Applicable.
√* = Data are available for an analog chemical (MCA) or hydrolysis product (DCA).

Physical and Chemical Characteristics

DCAC is a colorless liquid with an acrid, penetrating odor. DCAC hydrolyzes in water to form DCA and HCl. DCAC boils at 108°C, has a vapor pressure of 23 mm Hg @ 25°C, density of 1.53 @ 16/4°C, and a flash point of 66°C.

DCA is soluble in water. DCA has a melting point of 13.5°C, boiling point of 193-194°C, vapor pressure of 0.179 mm Hg @ 25°C, and density of 1.57 @ 13°C.

**Data for physical and chemical characteristics are complete and no further testing is recommended.**

**Table 2: Physical and Chemical Characteristics**

| | **DCAC** | **DCA** |
|---|---|---|
| **Melting Point** | N/A | 13.5°C |
| **Boiling Point** | 108°C | 193-194°C |
| **Density** | 1.53 @ 16/4°C | 1.57 @ 13°C |
| **Vapor Pressure** | 23 mm Hg @ 25°C | 0.179 mm Hg @ 25°C |
| **Log Kow** | -0.04 (estimated) | 0.92 (measured) |
| **Water Solubility** | Hydrolyzes to DCA and HCl | > 100 g/L |
| **Flash Point** | 66°C | No Data |

Environmental Fate

Both dichloroacetyl chloride and dichloroacetic acid, the major organic hydrolysis product, will tend to exist in the vapor phase in the atmosphere, because they both have a vapor pressure greater than 0.01 mm Hg at 25$^{o}$C. Dichloroacetyl chloride that becomes vaporized and is not contacted by liquid water is subject to hydroxyl radical oxidation with an estimated half-life of 855 days. A half-life of 22 days is estimated for hydroxyl radical oxidation of vapor phase dichloroacetic acid. Dichloroacetyl chloride reacts rapidly upon contact with water (half-life substantially less than one second). The expected primary products from hydrolysis are dichloroacetic acid and hydrochloric acid (HCl). Environmental fate information for dichloroacetic acid indicates that it is soluble in water at > 100 g/L (SRC, n.d.). Dichloroacetic acid is estimated to have a Henry's Law constant of $3.52 \times 10^{-7}$ atm-m$^3$/mole, which indicates that it has little tendency to volatilize from water. The estimated BCF value is 3, so it will not tend to bioaccumulate. It is reported to have an environmental half-life in temperate freshwater of < 100 hours (Ellis et al., 2001). The MITI database (CERI) reports that it is readily biodegradable, reaching 97% ThOD in 14 days. These characteristics indicate that dichloroacetyl chloride and its major degradation product, dichloroacetic acid, will not be persistent in water. Based on Level III fugacity modeling, using the assumption of equal emissions to air, water, and soil, any residual dichloroacetic acid is expected to be primarily distributed in water and soil. **No further environmental fate testing is recommended.**

**Table 3: Environmental Fate**

| | DCAC | DCA |
|---|---|---|
| **Bioaccumulation (BCF)*** | Unstable in water | Low potential for bioaccumulation, BCF = 3 |
| **Biodegradation** | Unstable in water | Readily biodegradable, 97% of ThBOD in 14 days (MITI test) |
| **Fugacity*** | Air 15.3%<br>Water 49.5%[1]<br>Soil 35.1%<br>Sediment 0.096%<br>Assuming equal emissions to air, water, and soil.<br><br>[1] As implemented, the Level III model does not account for the rapid rate of hydrolysis. Such adjustments will tend to move the distribution away from water. | Air 4.05%<br>Water 38.8%<br>Soil 57.2%<br>Sediment 0.077%<br>Assuming equal emissions to air, water, and soil. |
| * Modeled data. | | |

Modeling of physical/chemical parameters (i.e., Kow) and aquatic toxicity was conducted to help provide insight into the behavior in the environment and the aquatic toxicity of DCAC. Syracuse Research Corporation models for estimating physical/chemical properties were used to estimate $\log_{10}$ Kow (Meylan and Howard, 1995) for subsequent use in the ECOSAR program (Table 4). ECOSAR (Meylan and Howard, 1999) was used to estimate the aquatic toxicity of DCAC to green algae, daphnids (planktonic freshwater crustaceans), and fish. ECOSAR predictions are based on actual toxicity test data for classes of compounds with similar modes of action. No empirical aquatic toxicity data exist for DCAC. It was not possible to use ECOSAR to estimate the aquatic toxicity of DCAC to either invertebrates or algae; however, it was possible to estimate the 96-hour $LC_{50}$ for fish. It also was possible to use ECOSAR to estimate the acute toxicity of DCA (dichloroacetic acid) and its analog, MCA (monochloroacetic acid). Empirical data were also available for both of these compounds. Based on the available estimated or empirical data for DCA and MCA, the aquatic toxicity of DCAC is expected to be of low to medium concern. The toxicity of the other hydrolysis product of DCAC, hydrochloric acid, is likely to cause aquatic toxicity similar to or greater than DCA. Based on the rapid hydrolysis of DCAC and the availability of empirical data for its hydrolysis products, **no additional aquatic toxicity testing in recommended**.

**Table 4: Aquatic Toxicity**

| | **DCAC** | **DCA** | **MCA** |
|---|---|---|---|
| **Log Kow** | -0.04 (E)* | 0.52 (E) | 0.34 (E) |
| **Toxicity to Fish** (96-hour $LC_{50}$ value) | 572 mg/L (E) | 23,528 mg/L (E)<br><br>100 mg/L<br>(24-hour $LC_{50}$, N) | 25,457 mg/L (E)<br><br>370 mg/L<br>(96-hour, M)<br><br>LOEC = 25 mg/L<br>(28-day, M) |
| **Toxicity to Invertebrates** ($EC_{50}$ value) | No ECOSAR estimate | 22,761 mg/L (48-hour, E)<br><br>23.0 mg/L (96-hour, N) | 24,323 mg/L (48-hour, E)<br><br>96 mg/L (24-hour, N)<br>77 mg/L (48-hour, N)<br>32 mg/L<br>(21-day NOEC, N) |
| **Toxicity to Algae** (96-hour $EC_{50}$ value) | No ECOSAR estimate | 13,067 mg/L (E)<br><br>29.8-264.3 mg/L<br>(14-day endpoints, M) | 13,820 mg/L (E)<br><br>0.028 mg/L (48-hour, N)<br>0.025 mg/L (72-hour, N)<br>1.8 mg/L (72-hour, N) |
| *E = estimated value, N = value based on nominal test concentrations, M = measured test concentrations | | | |

Mammalian Toxicology

Acute toxicity data exists for both DCAC and its hydrolysis product, DCA. Both chemicals are slightly toxic via the acute oral route with $LD_{50}$s ranging from 2460 – 5520 mg/kg. Both chemicals are low to moderately toxic via the acute dermal route with $LD_{50}$s ranging from 0.51-650 mL/kg. Both DCAC and DCA are severe skin and eye irritants. **No further acute toxicity testing is recommended.**

**Table 5: Acute Toxicity**

| | DCAC | DCA |
|---|---|---|
| **Oral $LD_{50}$** | 2460 mg/kg (rat) | 2820 - 4480 mg/kg (rat)<br>5520 mg/kg (mice) |
| **4-hour Inhalation $LC_{LO}$** | 2000 ppm | No Data |
| **Dermal $LD_{50}$** | 650 mL/kg | 0.51 mL/kg |
| **Dermal Irritation** | Severe | Severe |
| **Eye Irritation** | Severe | Severe |
| **Dermal Sensitization** | No Data | No Data |

Repeated Dose Toxicity

Repeated dose toxicity data exists for both DCAC and its hydrolysis product, DCA. DCAC produced nasal tumors in a repeated dose inhalation study. In repeated dose oral studies conducted with DCA, hematologic, liver, testicular, nervous system, and cardiovascular effects were observed.

In a 30-day repeated dose inhalation study in rats where rats were monitored post-exposure until they spontaneously died or were moribund, DCAC produced nasal tumors at 2 ppm. The NOEL for oncogenic effects was 1 ppm. DCAC was also tested for carcinogenicity in female mice by repeated skin application and repeated subcutaneous injection. DCAC did not show skin tumorigenicity in the repeated skin application tests when tested at dosages of 1.5 and 3.0 mg/administration. DCAC showed marginally significant incidences of papillomas and carcinomas when tested as an initiator (5/50 mice exhibited tumors). When tested via subcutaneous injection at 2.0 mg/administration, 4/50 mice exhibited tumors.

In a 3-month oral study, DCA produced mortality, hindlimb paralysis, and biochemistry effects in rats. Histopathology examinations revealed that brain and testes were the target organs. Brain lesions occurred mainly in the cerebrum and to a lesser extent in the cerebellum. DCA-treated males exhibited testicular germinal epithelial degeneration and aspermatogenic testes. The LOAEL in rats was 125 mg/kg (the lowest dose tested). In dogs, DCA produced mortality, paralysis, biochemistry effects, and a high incidence of ocular anomalies. Histopathology findings included the ocular lesions, as well as effects in the lungs, brain, prostate, and testes. The LOAEL in dogs was 50 mg/kg (the lowest dose tested). A NOAEL was not established in rats or dogs.

In another 90-day oral study in dogs, DCA produced mortality, bilateral conjunctivitis, posterior paresis, and biochemical effects. The microscopic exam revealed effects in the brain, liver, lung, pancreas, and testes. A NOAEL was not determined in this study. The LOAEL was 12.5 mg/kg.

In an oral drinking water carcinogenicity study in rats, DCA induced observable signs of toxicity in the nervous system, liver, and myocardium. However, treatment-related neoplastic lesions were observed only in the liver. Testicular interstitial cell tumors were seen. DCA was considered a hepatocellular carcinogen in the male F344 rat. The authors state a NOEL of 0.05 g/L for DCA carcinogenicity in this study. **No further repeated dose toxicity testing is recommended.**

**Table 6: Repeated Dose Toxicity**

| | **DCAC** | **DCA** |
|---|---|---|
| Repeated Dose Studies | 30-Day Inhalation Study: Nasal tumors at 2 ppm.<br><br>NOEL = 1 ppm (rats) | 3-Month Oral Study: Peripheral neuropathy, brain, ocular, prostate, liver, lung, pancreas, and/or testes effects LOAEL = 125 mg/kg (rats) and 12.5 mg/kg (dogs). NOAELs were not determined. |
| Oncogenicity Study | Skin tumors at 3.0 mg/administration in a dermal initiation/promotion assay.<br><br>NOEL not determined (mice). | Neoplastic lesions in the liver.<br><br>NOEL = 0.05 g/L (rats) |

Developmental Toxicity

No developmental toxicity data were available for DCAC. DCA was administered to rats during gestation days 6-15 at dosages of 0, 14, 140, 400, 900, 1400, 1900, and 2400 mg/kg. Maternal toxicity as evidenced by reduced body weight gains was observed at = 140 mg/kg. Maternal death was observed at = 1400 mg/kg. Lower fetal weight and length and increased soft tissue malformations (cardiovascular system and ascending aorta and right ventricle) were observed at = 140 mg/kg. Since the NOEL for both maternal and fetal effects was 14 mg/kg/day, DCA was not considered a unique developmental toxin. **No further developmental toxicity testing is recommended.**

Reproductive Toxicity

While no formal reproductive toxicity studies have been conducted on DCAC, pathological examination of the testes in the 30-day inhalation study did not reveal any compound-related effects. A 14-day oral gavage study of DCA in rats, on the other hand, revealed delayed spermiation, formation of atypical residual bodies, distorted sperm heads and acrosomes, decreases in percentage of motile sperm, increased numbers of fused epidiymal sperm, and decreased epididymal weight and sperm count. No effects on the sperm were observed at

18 mg/kg. Testicular effects were also apparent in the repeated dose tests described earlier. Testicular degenerative lesions were observed as low as 12.5 mg/kg in a 90-day oral study in dogs. **No reproductive toxicity testing is recommended.**

Genetic Toxicity

DCAC was positive in a microscreen prophage induction assay. DCAC was also positive in a bacterial reverse mutation assay using *Salmonella* TA100 when tested without metabolic activation, but was negative in TA100 with metabolic activation. Molecular analysis of *Salmonella* revertants indicated that DCAC primarily induced GC to AT transitions. In a second bacterial reverse mutation assay using *Salmonella* TA98 and TA100, DCAC was again positive without metabolic activation in TA100, but was negative with metabolic activation in TA100 and TA98, and negative without metabolic activation in TA98. No data on the clastogenicity of DCAC were available.

DCA was also positive in a microscreen prophage induction assay. Both negative and positive results have been observed in bacterial mutation assays in *Salmonella* and *E. coli.* DCA was negative without metabolic activation and positive with activation in a DNA repair test, negative and positive in mouse lymphoma tests, positive in a cytogenetics test, negative in an *in vitro* micronucleus test, and negative in a CHO test. When tested *in vivo*, DCA was positive and negative in micronucleus tests, positive for mutagenicity in mouse liver, negative in an 8-OH DNA adduct test, and positive in a DNA strand breaking test. **No genetic toxicity testing is recommended.**

**Table 7: GeneticToxicity**

| | **DCAC** | **DCA** |
|---|---|---|
| **Mutagenicity** | Mutagenic | Mutagenic |
| **Clastogenicity** | No Data | Clastogenic |

Conclusion

Adequate data are available to address all the required endpoints. A substantial body of data exists for DCAC *per se*. Where data are lacking on DCAC, reliable data are available for the hydrolysis product, DCA. The use of DCA data to supplement the existing mammalian toxicity data for DCAC is supported by the close similarity in molecular structure, similarity in physical/chemical properties, and the similarity in toxicity observed where data for both substances are available for comparison. DCAC is rapidly hydrolyzed in aqueous media (half-life in water is <0.22 seconds) to DCA and HCl. The use of DCA data for DCAC is consistent with the Agency's directive to HPV participants to maximize the use of scientifically appropriate data for related chemicals. Although some differences between DCA and DCAC may be expected, we believe these differences to be minimal and insufficient to warrant additional animal testing.

Justification for Isolated Intermediate Status[1]:

DCAC is manufactured at Mobile and shipped to a small number of customers. Transportation is by tank truck or ISO, which are dedicated to DCAC service. The manufacturing process is a closed system, dedicated to DCAC manufacture. The shipping containers are closed with vent gases sent to a scrubber. Overfill protection for the loading operation is provided by a mass meter and there is spill containment at the rail spot including catch pan and sump. Any spills are treated in on-site waste facilities. For operations involving potential for DCAC exposure (line breaks, sampling, loading and unloading) proper PPE includes use of a butyl suit and full-face respirator with an airline supply. A full-face mask with acid cartridge would also be acceptable. Fresh cartridges each time are recommended to avoid break-through.

The customers who receive DCAC use it as a chemical intermediate in synthesis of other products. Their manufacturing systems are closed. Spill containment is used at the unloading spots, and PPE worn to protect the operators. Stewardship reviews of the customer facilities are conducted by DuPont personnel to verify the standards and practices are maintained.

References for the Summary:

Ellis, D. A. et al. (2001). Chemosphere, 42(3):309-318.

Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92.

Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210.

SRC (Syracuse Research Corporation) (n.d.). (HSDB/6894).

[1] As defined by EPA guidance, an isolated intermediate is one in which there is controlled transport, i.e. to a limited number of locations within the same company or second parties that use the chemical in a controlled way as an intermediate with a well known technology.

APPENDIX A

## ROBUST SUMMARY FOR DICHLOROACETYL CHLORIDE

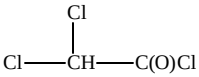
Existing published and unpublished data were collected and scientifically evaluated to determine the best possible study or studies to be summarized for each required endpoint. In the spirit of this voluntary program, other data of equal or lesser quality are not summarized, but are listed as related references at the end of each appropriate section, with a statement to reflect the reason why these studies were not summarized.

### 1.0 Substance Information

**CAS Number:** 79-36-7

**Chemical Name:** Acetyl chloride, dichloro-

**Structural Formula:**




**Other Names:** a, a-Dichloroacetyl chloride
Dichloroacetyl chloride
Dichloroacetic acid chloride
Dichloroethanoyl chloride

**Exposure Limits:** No information was found.

### 2.0 Physical/Chemical Properties

**2.1 Melting Point:** Not Applicable.

**2.2 Boiling Point**

| | |
|---|---|
| Value: | 108ºC |
| Decomposition: | No Data |
| Pressure: | No Data |
| Method: | No Data |
| GLP: | Unknown |
| Reference: | Lewis, R. J. (2000). Sax's Dangerous Properties of Industrial Chemicals, 10th ed., p. 1178-1179, John Wiley and Sons, Inc., New York. |
| Reliability: | Not assignable because limited study information was available. |

**Additional References for Boiling Point:**

DuPont Co. (2003). Material Safety Data Sheet No. MOB00519 (March 29).

Windholz, M. (1983). The Merck Index, 10th ed., p. 443, Merck and Co. Inc.,

Rahway, NJ.

## 2.3 Density

| | |
|---|---|
| Value: | density = 1.5315; vapor density = 5.8 |
| Temperature: | 16º/4ºC |
| Method: | No Data |
| GLP: | Unknown |
| Results: | No additional data. |
| Reference: | Lewis, R. J. (2000). Sax's Dangerous Properties of Industrial Chemicals, 10th ed., p. 1178-1179, John Wiley and Sons, Inc., New York |
| Reliability: | Not assignable because limited study information was available. |

**Additional References for Density:**

DuPont Co. (2003). Material Safety Data Sheet No. MOB00519 (March 29).

Windholz, M. (1983). The Merck Index, 10th ed., p. 443, Merck and Co. Inc., Rahway, NJ.

## 2.4 Vapor Pressure

| | |
|---|---|
| Value: | 23.02 mm Hg |
| Temperature: | 25ºC |
| Decomposition: | No Data |
| Method: | Measured |
| GLP: | Unknown |
| Reference: | Daubert, T. E. and R. P. Danner (1989). Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation, American Institute of Chemical Engineers, Hemisphere Pub. Corp., New York (NISC/EF-0004509). |
| Reliability: | Not assignable because limited study information was available. |

**Additional References for Vapor Pressure:**

DuPont Co. (2003). Material Safety Data Sheet No. MOB00519 (March 29).

Snelson, A. et al. (1978). A Study of Removal Processes for Halogenated Air Pollutants, US EPA-600/3-78-058 (HSDB/5229).

## 2.5 Partition Coefficient (log Kow)

Value: -0.04

| | |
|---|---|
| | Although a model estimate is made, it is indicated as "questionable" by the EPIWIN Software, because the extremely rapid hydrolysis makes the experimental estimation impractical. |
| Temperature: | 25°C |
| Method: | Modeled. KOWWIN, v. 1.67, module of EPIWIN 3.11 (Syracuse Research Corporation). KOWWIN uses "fragment constant" methodologies to predict log P. In a "fragment constant" method, a structure is divided into fragments (atom or larger functional groups) and coefficient values of each fragment or group are summed together to yield the log P estimate. |
| GLP: | Not Applicable |
| Reference: | Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92. |
| Reliability: | Estimated value based on accepted model. |

**Additional References for Partition Coefficient (log Kow):** None Found.

## 2.6 Water Solubility

| | |
|---|---|
| Value: | Unstable on contact with water. Dichloroacetyl chloride decomposes upon contact with water (Windholz, 1983). The rate of hydrolysis in a solution of 89.1% acetone and 10.9% water at -20ºC was experimentally determined to be 3.1 L/sec (Ugi and Beck, 1961), which corresponds to a half-life of 0.22 sec (SRC, n.d.). Hydrolysis products are expected to include HCl and dichloroacetic acid (SRC, n.d.). |
| Temperature: | -20ºC |
| pH/pKa: | Not Applicable |
| Method: | Conductometric detection of chloride release. |
| GLP: | Not Applicable |
| Reference: | Windholz, M. (1983). The Merck Index, 10th ed., p. 443, Merck and Co. Inc., Rahway, NJ (HSDB/5229).<br><br>Ugi, I. and F. Beck (1961). Chem. Ber., 94:1839.<br><br>SRC (n.d.). Syracuse Research Corporation (HSDB/5229). |
| Reliability: | Not assignable because limited study information was available. |

**Additional References for Water Solubility:**

DuPont Co. (2003). Material Safety Data Sheet No. MOB00519 (March 29).

Windholz, M. (1983). The Merck Index, 10th ed., p. 443, Merck and Co. Inc., Rahway, NJ.

### 2.7 Flash Point

| | |
|---|---|
| Value: | 66ºC |
| Method: | No Data |
| GLP: | Unknown |
| Reference: | Lewis, R. J. (2000). Sax's Dangerous Properties of Industrial Chemicals, 10th ed., p. 1178-1179, John Wiley and Sons, Inc., New York. |
| Reliability: | Not assignable because limited study information was available. |

**Additional Reference for Flash Point:**

Windholz, M. (1983). The Merck Index, 10th ed., p. 443, Merck and Co. Inc., Rahway, NJ.

### 2.8 Flammability

| | |
|---|---|
| Results: | Water hydrolyzes material liberating acidic gas which in contact with metal surfaces can generate flammable and/or explosive hydrogen gas. Emits toxic fumes under fire conditions. |
| Method: | No Data |
| GLP: | Unknown |
| Reference: | DuPont Co. (2003). Material Safety Data Sheet No. MOB00519 (March 29). |
| Reliability: | Not assignable because limited study information was available. |

**Additional References for Flammability:** None Found.

## 3.0 Environmental Fate

### 3.1 Photodegradation

| | |
|---|---|
| Concentration: | Not Applicable |
| Temperature: | Not Applicable |
| Direct Photolysis: | Not Applicable |
| Indirect Photolysis: | Estimated half-life of 855 days due to hydroxyl radical oxidation, assuming 24-hr day and a OH radical concentration of 0.5E6 OH/$cm^3$. |

Based upon data obtained from static reactor experiments at

| | |
|---|---|
| | 476 to 546ºK, the gas-phase hydrolysis half-life between water and dichloroacetyl chloride in the troposphere has been determined to be in excess of 100 years (Snelson et al., 1978). Atmospheric wash-out and transformation via rain may be possible (SRC, n.d.). |
| Breakdown Products: | Not Applicable |
| Method: | Indirect Photolysis: AOPWIN, v. 1.91 module of EPIWIN 3.11. |
| GLP: | Not Applicable |
| Reference: | Indirect Photolysis: AOPWIN, v.1.91 module of EPIWIN v3.11. Meylan, W. M. and P.H. Howard (1993). Chemosphere, 26:2293-99.<br><br>Snelson, A. et al. (1978). A Study of Removal Processes for Halogenated Air Pollutants, U.S. EPA-600/3-78-058 (HSDB/5229).<br><br>SRC (n.d.). Syracuse Research Corporation (HSDB/5229). |
| Reliability: | Estimated value based on accepted model. |

**Additional References for Photodegradation:** None Found.

### 3.2 Stability in Water

| | |
|---|---|
| Concentration: | Unstable on contact with water. Dichloroacetyl chloride decomposes upon contact with water (Windholz, 1983). The rate of hydrolysis in a solution of 89.1% acetone and 10.9% water at -20ºC was experimentally determined to be 3.1 L/sec (Ugi and Beck, 1961), which corresponds to a half-life of 0.22 sec (SRC, n.d.). Hydrolysis products are expected to include HCl and dichloroacetic acid (SRC, n.d.). |
| Half-life: | < 0.22 sec (reaction rate extrapolated to pure water is estimated to be much faster than in the acetone-water mixture, but it is impractical to measure such rapid rates (Ugi and Beck, 1961)). |
| % Hydrolyzed: | ~100% |
| Method: | Conductometric detection of chloride release. |
| GLP: | Not Applicable |
| Reference: | Windholz, M. (1983). The Merck Index, 10th ed., p. 443, Merck and Co. Inc., Rahway, NJ (HSDB /5229).<br><br>Ugi, I. and F. Beck (1961). Chem. Ber., 94:1839.<br><br>SRC (n.d.). Syracuse Research Corporation (HSDB/5229). |
| Reliability: | Not assignable because limited study information was |

available.

**Additional References for Stability in Water:** None Found.

### 3.3 Transport (Fugacity):

Media: Air, Water, Soil, and Sediments

Distributions:

| Compartment | % of total distribution | ½ life hours (advection + reaction)* |
|---|---|---|
| Air | 15.3 | 20,500 |
| Water | 49.5 | 900 |
| Soil | 35.1 | 1800 |
| Sediment | 0.096 | 8100 |

* - This implementation of the Level III fugacity model fails to incorporate hydrolysis rates into distribution calculations. Such adjustments will tend to move the distribution away from water.

Adsorption Coefficient: Koc = 0.374 (calc by model)

Desorption: Not Applicable

Volatility: Henry's Law Constant = 8.25e-005 atm-$m^3$/mole (HENRYWIN program)

Method: Environmental Distribution - Mackay Level III fugacity model, in EPIWIN v3.11 (Syracuse Research Corporation). Emissions (1000 kg/hr) to air, water, and soil compartments.

Data Used
SMILES: O=C(C(CL)CL)CL
Vapor Pressure: 23.02 mm Hg (experimental)
Log Kow: -0.04 (KOWWIN program)

Henry's Law Constant - HENRYWIN v. 3.10 module of EPIWIN v3.11 (Syracuse Research Corporation). Henry's Law Constant (HLC) is estimated by two separate methods that yield two separate estimates. The first method is the bond contribution method and the second is the group contribution method. The bond contribution method is able to estimate many more types of structures; however, the group method estimate is usually preferred (but not always) when all fragment values are available.

Log Koc – Calculated from log Kow by the Mackay Level III fugacity model incorporated into EPIWIN v3.11 (Syracuse Research Corporation).

GLP: Not Applicable

Reference: HENRYWIN –

Hine. J. and P. K. Mookerjee (1975). J. Org. Chem., 40(3):292-298.

Meylan, W. and P. H. Howard (1991). Environ. Toxicol. Chem., 10:1283-1293.

Fugacity - The methodology and programming for the Level III fugacity model incorporated into EPIWIN v3.11 (Syracuse Research Corporation) were developed by Dr. Donald MacKay and coworkers and are detailed in:

Mackay, D. (1991). Multimedia Environmental Models: The Fugacity Approach, pp. 67-183, Lewis Publishers, CRC Press.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.

Reliability: Estimated values based on accepted models.

**Additional References for Transport (Fugacity):** None Found.

### 3.4 Biodegradation:

| | |
|---|---|
| Value: | Because of rapid hydrolysis, half-life in water is < 1 sec; measuring biodegradation rates for this substance is not practical. |
| Linear Model Prediction: | 0.455 – Does not biodegrade fast |
| Non-Linear Model Prediction: | 0.057 - Does not biodegrade fast |
| Ultimate Biodegradation Timeframe: | 2.527 – weeks to months |
| Primary Biodegradation Timeframe: | 3.434 – days to weeks |
| MITI Linear Model Prediction: | 0.225 – Not readily biodegradable |
| MITI Non-Linear Model Prediction: | 0.0428 – Not readily biodegradable |

| | |
|---|---|
| Breakdown Products: | No Data |
| Method: | Modeled. BIOWIN, v.4.01 module of EPINWIN v3.11 (Syracuse Research Corporation). BIOWIN estimates the probability for the rapid aerobic biodegradation of an organic chemical in the presence of mixed populations of environmental microorganisms. Estimates are based upon fragment constants that were developed using multiple linear and non-linear regression analyses. |
| GLP: | Not Applicable |
| Reference: | Boethling, R. S. et al. (1994). Environ. Sci. Technol., 28:459-65.<br><br>Howard, P. H. et al. (1992). Environ. Toxicol. Chem., 11:593-603.<br><br>Howard, P. H. et al. (1987). Environ. Toxicol. Chem., 6:1-10.<br><br>Tunkel, J. et al. (2000). Predicting Ready Biodegradability in the MITI Test. Environ. Toxicol. Chem., accepted for publication. |
| Reliability: | Estimated value based on accepted model. |

**Additional References for Biodegradation:** None Found.

### 3.5 Bioconcentration

| | |
|---|---|
| Value: | Dichloroacetyl chloride hydrolyzes on contact with water (Windholz, 1983), therefore, bioconcentration in aquatic organisms is not possible (SRC, n.d.). Dichloroacetic acid, the expected primary organic breakdown product has an estimated BCF of 3 (SRC, n.d.). |
| Method: | Modeled. BCFWIN v2.15 module of EPIWIN v3.11 (Syracuse Research Corporation). BCFWIN estimates the bioconcentration factor (BCF) of an organic compound using the compound's log octanol-water partition coefficient (Kow) with correction factors based on molecular fragments. |
| GLP: | Not Applicable |
| Reference: | Windholz, M. (1983). The Merck Index, 10th ed., p. 443, Merck and Co. Inc., Rahway, NJ (HSDB/5229).<br><br>SRC (n.d.). Syracuse Research Corporation (HSDB/5229).<br><br>"Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient", |

| | |
|---|---|
| | SRC TR-97-006 (2nd Update), July 22, 1997; prepared for: Robert S. Boethling, EPA-OPPT, Washington, DC; Contract No. 68-D5-0012; prepared by: William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup and Sybil Gouchie; Syracuse Research Corp. |
| Reliability: | Estimated value based on accepted model. |

**Additional References for Bioconcentration:** None Found.

## 4.0 Ecotoxicity

### 4.1 Acute Toxicity to Fish:

| | |
|---|---|
| **Type:** | **96-Hour $LC_{50}$** |
| Species: | Fish |
| Value: | 572 mg/L (using Log Kow of –0.04) |
| Method: | Modeled |
| GLP: | Not Applicable |
| Test Substance: | DCAC |
| Results: | No additional data. |
| Reference: | Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication). |
| Reliability: | Estimated value based on accepted model. |

**Supporting Data: DCA –** See Appendix B

**Supporting Data: MCA**

| | |
|---|---|
| **Type:** | **96-Hour $LC_{50}$** |
| Species: | *Branchydanio reiro*, zebrafish |
| Value: | 370 mg/L |
| Method: | No information was provided. |
| GLP: | Unknown |
| Test Substance: | MCA, purity not reported |
| Results: | pH was neutral. |
| Reference: | CIT (Centre International de Toxicologie) (1998). Unpublished data, Study No. 16198 ECP, "Monochloroacetic acid. Early-life stage toxicity test in *Brachydanio rerio* under semi-static conditions" (cited in ECETOC (1999). Joint Assessment of Commodity Chemicals No. 38, "Monochloroacetic acid and its sodium |

| | |
|---|---|
| | salt" (June)). |
| Reliability: | ECETOC gave this study a reliability of 1a (reliable without restriction). |

| | |
|---|---|
| **Type:** | **28-Day LOEC (Early life stage)** |
| Species: | *Branchydanio reiro*, zebrafish |
| Value: | 25 mg/L |
| Method: | The procedures used in the test were based on the recommendations of the following guideline: OECD Guideline 210. Concentrations tested ranged from 25-400 mg/L. |
| GLP: | Unknown |
| Test Substance: | MCA, purity not reported |
| Results: | pH was neutral. No NOEC was found. When the control mortality was subtracted from the data set, 15% mortality was found at 25 mg/L (the lowest concentration tested). |
| Reference: | CIT (Centre International de Toxicologie) (1998). Unpublished data, Study No. 16198 ECP, "Monochloroacetic acid. Early-life stage toxicity test in *Brachydanio rerio* under semi-static conditions" (cited in ECETOC (1999). Joint Assessment of Commodity Chemicals No. 38, "Monochloroacetic acid and its sodium salt" (June)). |
| Reliability: | ECETOC gave this study a reliability of 1b (comparable to guideline study). |

| | |
|---|---|
| **Type:** | **96-Hour $LC_{50}$** |
| Species: | Fish |
| Value: | 25,457 mg/L (using Log Kow of 0.34) |
| Method: | Modeled |
| GLP: | Not Applicable |
| Test Substance: | MCA |
| Results: | No additional data. |
| Reference: | Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication). |
| Reliability: | Estimated value based on accepted model. |

**Additional References for MCA:**

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were

not substantially additive to the database.

AKZO (1985). Unpublished Data, Mileu risicobeoordeling van het monochloorazijnsuur-destillatieresidu, afkomstig van AZC-Hengelo. Acute toxiciteitstest voor waterdieren (NEN 6504: *Poecilia reticulata* – NEN 6501: *Daphnia magna*) (cited in ECETOC (1999). Joint Assessment of Commodity Chemicals No. 38, "Monochloroacetic acid and its sodium salt" (June)).

Hoechst AG (1979). Unpublished data 79.0399 (cited in ECETOC (1999). Joint Assessment of Commodity Chemicals No. 38, "Monochloroacetic acid and its sodium salt" (June)).

McCarty, W. M. et al. (1977). Dow Europe, Unpublished Report or Communication (cited in OECD SIDS Dossier for Chloroacetic Acid, accessed via http://cs3-hq.oecd.org/scripts/hpv/ on April 6, 2004).

Hoechst AG (1992). Unpublished Data (cited in OECD SIDS Dossier for Chloroacetic Acid, accessed via http://cs3-hq.oecd.org/scripts/hpv/ on April 6, 2004).

Loeb, H. A. and W. H. Kelly (1963). US Fish Wildl. Serv., Sp. Sci. Rep. – Fish No. 471, p. 124, Washington, DC (AQUIRE/AQ-0063263 - AQ-0063265).

US EPA (1993). AQUIRE (cited in OECD SIDS Dossier for Chloroacetic Acid, accessed via http://cs3-hq.oecd.org/scripts/hpv/ on April 6, 2004).

Applegate, V. C. et al. (1957). US Fish Widl. Serv., Spec. Sci. Rep. Fish No. 207, p. 157, USDI, Washington, DC (AQUIRE/AQ-0030805 - AQ-0030806).

Walterson, E. et al. (1980). Monklorattiksyra: Toxikologisk Dokumentation Samt Preliminar Bedomning av Effekter I Recipienten Inst. for Vatten- och Luftvardsforskning, R 41/80 (cited in OECD SIDS Dossier for Chloroacetic Acid, accessed via http://cs3-hq.oecd.org/scripts/hpv/ on April 6, 2004).

Meinck, F. et al. (1970). Les Eaux Residuaires Industrielles, 2nd ed. (cited in OECD SIDS Dossier for Chloroacetic Acid, accessed via http://cs3-hq.oecd.org/scripts/hpv/ on April 6, 2004).

## 4.2 Acute Toxicity to Invertebrates:

**Supporting Data: DCA –** See Appendix B

**Supporting Data: MCA**

| | |
|---|---|
| **Type:** | **48-hour $EC_{50}$** |
| Species: | *Daphnia magna* Straus |

Value: 77 mg/L (95% confidence limits, 71-85 mg/L)

Method: The procedures used in the test were based on the recommendations of the following guideline: DIN 38412, Part II.

The daphnids were 6-24 hours old.

The dilution water had the following characteristics: acid capacity $K_{S4.3}$ of 0.8 mmol/L, total hardness of 2.4 mmol/L, a calcium to magnesium ratio of 4:1, a sodium to potassium ratio of 10:1, and a pH of 8.0±0.2.

Test solutions were kept at 20ºC in an incubator. Test periods lasted 24 and 48 hours. No food was given during the test period.

From the dilution with the pollutant, dilution series were prepared using the dilution water. Concentrations of the test solution were selected to give 3-4 EC values in a range between $EC_0$ and $EC_{100}$, with at least 1 value below and 1 above the $EC_{50}$. Actual concentrations were not reported. Two 50 mL beakers were used for test vessels. Two parallel preparations were used for each concentration with 10 daphnids tested in each preparation, totaling 20 daphnids per concentration.

After 24 and 48 hours, the number of daphnids that could swim was counted. At 48 hours, the pH and oxygen concentration were also measured.

The test was considered valid if fewer than 10% of the animals in the control solution were unable to swim, if the pH value was not below 7.0, and if the $O_2$ value was not below 4.0 mg/L.

The $EC_0$ and $EC_{100}$ values were taken from the results obtained from the test solutions. The 24- and 48-hour $EC_{50}$s were calculated arithmetically from the concentration/effect ratio.

GLP: Unknown

Test Substance: MCA, purity not reported

Results: 24-hour $EC_{50}$ = 99 mg/L (95% confidence intervals, 94-104 mg/L)
24-hour $EC_0$ = 81 mg/L
24-hour $EC_{100}$ = 107 mg/L

48 hour $EC_0$ = 55 mg/L
48-hour $EC_{100}$ = 107 mg/L

Reference: Kuhn, R. et al. (1989). Water Res., 23(4):495-499.

Reliability: Medium because a suboptimal study design (nominal concentrations only) was used.

**Type:** **24-hour $EC_{50}$ and 21-day Reproduction Test**

Species: *Daphnia magna* Straus

Value: 24-hour $EC_{50}$ = 96 mg/L for immobilization

21-day NOEC = 32 mg/L for reproductive effects

Method: The procedures used in the test were based on the recommendations of the following guideline: Provisional procedure proposed by the Federal Environmental Agency (Umweltbundesamt) as of 1 January 1984.

The test substance was dissolved (both quantitatively and optically) in dilution water using magnetic stirrers. A stock solution of 400 mg/L MCA was prepared for the reproduction test. Test concentrations ranged from 0.032-100 mg/L. Test vessels were 400 mL beakers with a 250 mL useful capacity. Four parallel test vessels per concentration level and control were filled with 5 (24 hour-old) *Daphnia*. The total number of daphnids used per test concentration was 20.

A semi-static test method was adopted, in which the parent animals in the test and control vessels were pipetted 3 times per week into freshly prepared test and control media at the corresponding test concentrations. At these times, dead parent animals or those incapable of swimming were removed. Offspring were counted and the total number of offspring per vessel was recorded.

The pH and oxygen concentration were measured in 2 test vessels per concentration level. Samples were taken twice from selected concentration levels and analyzed chemically.

The Student's t-test and the U-test were used to statistically analyze the data and determine NOEC's.

GLP: Unknown

Test Substance: MCA, purity not reported

Results: The 24-hour $EC_0$ = 85 mg/L (nominal value).

On no occasion was the pH value (based on 8.0±0.2) lower than 7.0. It remained in the neutral to subalkaline range.

| | |
|---|---|
| | The average minimum oxygen saturation was 69% at the end of the test period. |
| Reference: | Kuhn, R. et al. (1989). Water Res., 23:501-510. |
| Reliability: | Medium because a suboptimal study design (nominal concentrations only) was used. |

| | |
|---|---|
| **Type:** | **48-hour $EC_{50}$** |
| Species: | Daphnid |
| Value: | 24,323 mg/L (using log Kow of 0.34) |
| Method: | Modeled |
| GLP: | Not Applicable |
| Test Substance: | MCA |
| Results: | No additional data. |
| Reference: | Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication). |
| Reliability: | Estimated value based on accepted model. |

**Additional References for MCA:**

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Bundesminister des Innern (1982). Environmental Research Plan, 15, 20, 21, 25, 28, 33 (July) (cited in IUCLID (2000). Data Set "chloroacetic acid" (February 19)).

Hoechst AG (1992). Unpublished Data (cited in OECD SIDS Dossier for Chloroacetic Acid, accessed via http://cs3-hq.oecd.org/scripts/hpv/ on April 6, 2004).

Bautonett, J. C. (1988). ATOCHEM (cited in OECD SIDS Dossier for Chloroacetic Acid, accessed via http://cs3-hq.oecd.org/scripts/hpv/ on April 6, 2004).

AKZO (1985). Unpublished Data, Mileu risicobeoordeling van het monochloorazijnsuur-destillatieresidu, afkomstig van AZC-Hengelo. Acute toxiciteitstest voor waterdieren (NEN 6504: *Poecilia reticulata* – NEN 6501: *Daphnia magna*) (cited in ECETOC (1999). Joint Assessment of Commodity Chemicals No. 38, "Monochloroacetic acid and its sodium salt" (June)).

McCarty, W. M. et al. (1977). Dow Europe, Unpublished Report or Communication (cited in OECD SIDS Dossier for Chloroacetic Acid, accessed via http://cs3-hq.oecd.org/scripts/hpv/ on April 6, 2004).

Meinck, F. et al. (1970). Les Eaux Residuaires Industrielles, 2nd ed. (cited in OECD SIDS Dossier for Chloroacetic Acid, accessed via http://cs3-hq.oecd.org/scripts/hpv/ on April 6, 2004).

**4.3 Acute Toxicity to Aquatic Plants:** No Data.

**Supporting Data: DCA –** See Appendix B

**Supporting Data: MCA**

| | |
|---|---|
| **Type:** | **48-hour $EC_{50}$ Cell Multiplication Inhibition Test** |
| Species: | Green algae, *Scenedesmus subspicatus* |
| Value: | 0.028 mg/L (impact on biomass) |
| Method: | The procedures used in the test were based on the recommendations of the following guideline: DIN 38 412, Part 1, 1982. |

The test substance was dissolved (both quantitatively and optically) in dilution water using magnetic stirrers. A stock solution of MCA was prepared and from this test concentrations ranging from 0.0008-1.0 mg/L were prepared. Stock solutions were adjusted to pH 8.0±0.3. Test vessels consisted of 250 mL wide-necked bottles with glass stoppers. Test and control preparations were incubated under constant lighting and shaken daily.

The extinction value of the monochromatic radiation of the cell suspension was determined at the beginning of the test and after 24 and 48 hours. Determination of biomass was via the measurement of optical density (measurement of turbidity). After determining the optical density, pH was also measured.

Growth curves were established for each tested concentration and the control. The area under the growth curves was calculated, and from this was calculated the percentage inhibition of cell multiplication on the basis of a comparison of the biomass (B) formed under the influence of the test substance with the biomass in the control preparation. The average growth rate was calculated for cultures showing exponential growth. The growth-related inhibition (μ) was calculated on this basis. The tested

| | |
|---|---|
| | concentration was assigned to the respective inhibition values in the probability paper. The regression line was determined, and from this the desired values of $E_BC_{10}$ and $E_BC_{50}$ and/or $E_\mu C_{10}$ and $E_\mu C_{50}$ were read. |
| GLP: | Unknown |
| Test Substance: | MCA, purity not reported |
| Results: | $E_BC_{10}$ (0-48 hours) = 0.007 mg/L<br>$E_BC_{50}$ (0-48 hours) = 0.028 mg/L<br>$E_\mu C_{10}$ (0-48 hours) = 0.014 mg/L<br>$E_\mu C_{50}$ (0-48 hours) =0.07 mg/L |
| Reference: | Kuhn, R. and M. Pattard (1990). Water Res., 24:31-38. |
| Reliability: | Medium because a suboptimal study design (nominal concentrations only) was used. |

| | |
|---|---|
| **Type:** | **72-hour $EC_{50}$ Biomass Test** |
| Species: | Green algae, *Scenedesmus subspicatus* |
| Value: | 0.025 mg/L (impact on biomass) |
| Method: | The procedures used in the test were based on the recommendations of the following guideline: OECD 201. |
| GLP: | Yes |
| Test Substance: | MCA, purity not reported |
| Results: | NOEC = 0.0058 mg/L (biomass)<br>$EC_{10}$ = 0.006 mg/L (biomass)<br><br>$ErC_{50}$ = 0.033 mg/L<br>$ErC_{10}$ = 0.007 mg/L<br>$ErC_0$ (NOEC) = 0.0058 mg/L<br><br>pH was neutral. |
| Reference: | Hoechst AG (1992). Unpublished Data, SZO 24641 (cited in ECETOC (1999). Joint Assessment of Commodity Chemicals No. 38, "Monochloroacetic acid and its sodium salt" (June)). |
| Reliability: | ECETOC gave this study a reliability of 1b. Comparable to guideline study. |

| | |
|---|---|
| **Type:** | **72-hour $ErC_{50}$ (Growth Rate)** |
| Species: | Green algae, *Selenastrum capricornutum* |
| Value: | 1.8 mg/L |
| Method: | No information was available. |
| GLP: | Unknown |
| Test Substance: | MCA, purity not reported |
| Results: | $ErC_{20}$ = 0.13 mg/L<br>$ErC_{10}$ = 0.06 mg/L<br>LOEC = 0.005 mg/L<br>NOEC < 0.005 mg/L |

| | |
|---|---|
| Reference: | Eka Nobel (1993). Unpublished data, "Monochloroacetic acid algal growth inhibition test. Vattenvardslaboriet No. 04-14" (cited in ECETOC (1999). Joint Assessment of Commodity Chemicals No. 38, "Monochloroacetic acid and its sodium salt" (June)). |
| Reliability: | ECETOC gave this study a reliability of 3a. Documentation insufficient for assessment. |

**Additional Reference for MCA:**

Data from this additional source supports the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Bundesminister des Innern (1982). Environmental Research Plan, 15, 20, 21, 25, 28, 33 (July) (cited in IUCLID (2000). Data Set "chloroacetic acid" (February 19)).

## 5.0 Mammalian Toxicity

### 5.1 Acute Toxicity

| | |
|---|---|
| **Type:** | **Oral $LD_{50}$** |
| Species/Strain: | Rats/Paper indicated that the methods were basically the same as earlier studies by these authors. These studies used the Wistar or Sherman strains. |
| Value: | 2460 mg/kg (1830-3230 mg/kg) |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>Four groups of 5 non-fasted male rats were given single oral dosages of DCAC. Although the 4 dosages used were not given, they were in a geometrical series such as 1, 2, 4, 8 g/kg. The $LD_{50}$ with standard deviations was calculated by the method of Thompson. |
| GLP: | No |
| Test Substance: | DCAC, purity not reported |
| Results: | No additional data. |
| Reference: | Smyth, H. F., Jr. et al. (1951). Arch. Ind. Hyg. Occup. Med., 4:119.<br><br>Smyth, H. F., Jr. et al. (1949). J. Indust. Hyg. Toxicol., 31:60. |
| Reliability: | High because a scientifically defensible or guideline method was used. |

| | |
|---|---|
| **Type:** | **Oral ALD** |
| Species/Strain: | Male rats/Albino |
| Value: | 1000 mg/kg |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>One male rat/group was administered 200, 300, 450, 670, 1000, 1500, or 2250 mg/kg DCAC in peanut oil. Body weights and clinical signs were recorded periodically over the 12-day observation period. Gross autopsies were performed on all rats and pancreas, spleen, liver, stomach, adrenal, kidney, and/or genitals were saved for microscopic examination. |
| GLP: | No |
| Test Substance: | DCAC, purity not reported |
| Results: | Mortality was observed in the 1000 and 2250 mg/kg groups.<br><br>Clinical signs included weakness in hind legs, paleness, and labored respiration. The principal pathologic finding was acute gastritis. Other observations included hepatitis and congestion of the pancreas and spleen. In 1 animal receiving the highest dose administered, 2250 mg/kg, the chemical appeared to diffuse through the stomach wall to the surrounding viscera and adjacent blood vessels. |
| Reference: | DuPont Co. (1955). Unpublished Data, Haskell Laboratory Report No. 51-55, "Toxicity of Dichloroacetyl chlorides" (September 15). |
| Reliability: | High because a scientifically defensible or guideline method was used. |

**Additional References for Acute Oral Toxicity:** None Found.

| | |
|---|---|
| **Type:** | **Inhalation $LC_{Lo}$** |
| Species/Strain: | Rats/Paper indicated that the methods were basically the same as earlier studies by these authors. These studies used the Wistar or Sherman strains. |
| Exposure Time: | 4 hours |
| Value: | 2000 ppm |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>Groups of 6 male albino rats were exposed to a flowing stream of air substantially saturated with vapors of DCAC. |

| | |
|---|---|
| | Creation of a mist was obtained by bubbling air through the sample held at 170ºC, cooling the air, and furnishing it to the animals in a small chamber. Known concentrations were used, aiming at a concentration which produced fractional mortality as a result of a 4-hour exposure. Concentrations used fell in a series differing by a ratio of 2, and they were approximations because they were estimated from the settings of a proportional pump and flowmeter, rather than being determined analytically. |
| GLP: | No |
| Test Substance: | DCAC, purity not reported |
| Results: | At 2000 ppm, 2/6 rats died.<br><br>The maximum exposure time to saturated vapor which produced no deaths was 8 minutes. |
| Reference: | Smyth, H. F., Jr. et al. (1951). Arch. Ind. Hyg. Occup. Med., 4:119.<br><br>Smyth, H. F., Jr. and C. P. Carpenter (1948). J. Indust. Hyg. Toxicol., 30:63. |
| Reliability: | High because a scientifically defensible or guideline method was used. |

**Additional References for Acute Inhalation Toxicity:** None Found.

| | |
|---|---|
| **Type:** | **Dermal $LD_{50}$** |
| Species/Strain: | Rabbits/Albino |
| Exposure Time: | 24 hours |
| Value: | 650 mL/kg (530-810 mL/kg) |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>Undiluted DCAC was applied to the clipped skin of the rabbit trunk using a modification of the rubber cuff of the FDA (Draize et al. (1944). J. Pharmacol. Exper. Therap., 82:377). The dose was retained under a flexible film of rubber, vinyl plastic, or the like, selected to be impervious to the chemical. Dosages up to 20 mL/kg may have been used. The number of animals used per dosage was 5. The $LD_{50}$ with standard deviations was calculated by the method of Thompson. |
| GLP: | No |
| Test Substance: | DCAC, purity not reported |
| Results: | No additional data. |
| Reference: | Smyth, H. F., Jr. et al. (1951). Arch. Ind. Hyg. Occup. Med., |

4:119.

Smyth, H. F., Jr. and C. P. Carpenter (1944). J. Indust. Hyg. Toxicol., 26:269.

Smyth, H. F., Jr. and C. P. Carpenter (1948). J. Indust. Hyg. Toxicol., 30:63.

Reliability: High because a scientifically defensible or guideline method was used.

**Additional References for Acute Dermal Toxicity:** None Found.

**Type:** **Dermal Irritation**

Species/Strain: Rabbits/Albino

Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

An undiluted sample (0.01 mL) of DCAC was applied to an area upon the clipped belly of a rabbit. The rabbit was observed after 24 hours, recording necrosis, edema, erythema, or congestion of capillaries. If the undiluted material gave evidence of strong primary irritation, it was applied in the form of a 1% solution in acetone to locate the least concentration causing irritation.

Hazard from primary irritation was expressed in numerical grades, based on the reactions of 5 rabbits, scored somewhat similarly to the method of Draize.

Grade 1 showed no reaction whatever from the undiluted sample.
Grade 2 showed an average reaction equivalent to a trace of capillary injection.
Grade 3 showed strong capillary injection.
Grade 4 showed slight erythema.
Grade 5 showed strong erythema, edema, or slight necrosis.
Grade 6 was used if a 10% acetone solution gave no reaction more severe than edema.
Grade 7 was used if a 1% acetone solution gave no reaction more severe than edema.
Grade 8 was used if a 0.1% acetone solution gave no reaction more severe than edema.
Grade 9 was used if a 0.01% acetone solution gave no reaction more severe than edema.
Grade 10 was used if a weaker solution was determined to

| | |
|---|---|
| | give no reaction more severe than edema. |
| GLP: | No |
| Test Substance: | DCAC, purity not reported |
| Results: | A 1% solution in acetone produced edema. The primary skin irritation score for rabbits in the study was determined to be a 7. |
| Reference: | Smyth, H. F., Jr. et al. (1951). Arch. Ind. Hyg. Occup. Med., 4:119.<br><br>Smyth, H. F., Jr. and C. P. Carpenter (1944). J. Indust. Hyg. Toxicol., 26:269.<br><br>Smyth, H. F., Jr. et al. (1949). J. Indust. Hyg. Toxicol., 31:60. |
| Reliability: | Medium because a suboptimal study design was used. |

| | |
|---|---|
| **Type:** | **Dermal Irritation** |
| Species/Strain: | Rabbits/Not specified |
| Method: | No specific test guideline was reported. Secondary source reports that 2 mg was tested. Test duration was 24 hours. |
| GLP: | Unknown |
| Test Substance: | DCAC, purity not reported |
| Results: | Severe |
| Reference: | Anon. (1986). Prehled Prumysloe Toxikolgie; Organicke Latky, p. 571 (cited in Lewis, R. J. (2000). Sax's Dangerous Properties of Industrial Chemicals, 10th ed., p. 1178-1179, John Wiley and Sons, Inc., New York). |
| Reliability: | Not assignable because limited study information was available. |

**Additional Reference for Dermal Irritation:**

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Hawley G. G. (1977). The Condensed Chemical Dictionary, 9th ed., p. 277, Van Nostrand Rehinold, Co., New York (HSDB/5229).

**Type:** **Dermal Sensitization:** No Data.

| | |
|---|---|
| **Type:** | **Eye Irritation** |
| Species/Strain: | Rabbits/Albino |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. |

| | |
|---|---|
| | Measured volumes of undiluted DCAC were placed on the center of the cornea of an albino rabbit which was shown previously to have uninjured eyes. After 24 hours, the eye was observed for gross evidence of injury and for corneal necrosis revealed by fluorescein stain. Volumes used were 0.001, 0.005, 0.02, 0.1, and 0.5 mL. The actual volume used was based on previous experience. Not all volumes were tested.<br><br>In some cases, an excess of a solution of the chemical in a non-irritating solvent, such as water or propylene glycol, was also used. The concentration used was selected from the series 40, 15, 5, 1, and 0.1%.<br><br>The scoring system was described in Carpenter, C. P. and H. F. Smyth, Jr. (1946). Am. J. Ophth., 29:1363. An injury grade of 10 was given when an excess of a 1% solution gave an injury of 5.0 points. A score of 5.0 points was described as a severe injury. |
| GLP: | No |
| Test Substance: | DCAC, purity not reported |
| Results: | The eye injury in rabbits was given an injury grade of 10. |
| Reference: | Smyth, H. F., Jr. et al. (1951). Arch. Ind. Hyg. Occup. Med., 4:119.<br><br>Smyth, H. F., Jr. and C. P. Carpenter (1944). J. Indust. Hyg. Toxicol., 26:269. |
| Reliability: | High because a scientifically defensible or guideline method was used. |

**Additional References for Eye Irritation:**

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (2003). Material Safety Data Sheet No. MOB00519 (March 29).

Hawley G. G. (1977). The Condensed Chemical Dictionary, 9th ed., p. 277, Van Nostrand Rehinold, Co., New York (HSDB/5229).

## 5.2 Repeated Dose Toxicity

| | |
|---|---|
| **Type:** | **30-Day Inhalation Study** |
| Species/Strain: | Rats/Sprague-Dawley |
| Sex/Number: | Male/50 per DCAC group or 98 in the control group |
| Exposure Period: | 30 days |
| Frequency of Treatment: | 6 hours/day, 5 days/week |
| Exposure Levels: | 0, 0.5, 1.0, 2.0 ppm |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>Male rats were approximately 9-10 weeks old and weighed 325±16.8 g at study start. Food and water were provided *ad libitum* except during inhalation exposures.<br><br>Test atmospheres were generated by passing an airstream over the liquid test substance in a generating flask and then feeding the effluent vapor into the chamber air supply. Animal exposures were done in 1.0 $m^3$ or 1.3 $m^3$ dynamic exposure chambers. Chamber concentrations were measured periodically throughout the exposure and analyzed via an infrared gas analyzer.<br><br>All animals were observed daily and weighed monthly. All animals were allowed to die spontaneously or were sacrificed when moribund. Complete necropsies were performed at sacrifice. Nasal passages, brain, lung, trachea, larynx, liver, kidney, testes, and any other organs exhibiting gross pathology were examined microscopically. Duration of the post-exposure period extended out to approximately 128 weeks. |
| GLP: | Unknown |
| Test Substance: | DCAC, purity > 95% |
| Results: | The mean exposure concentrations for the 0.5, 1.0, and 2.0 ppm groups were 0.53, 1.03, and 2.00 ppm, respectively.<br><br>Nasal tumors were present at 2 ppm. Two of 50 animals had squamous cell carcinoma or mixed cell carcinoma of nasal mucosa. These animals died 701 and 887 days after the initial exposure. No tumors were observed in the controls.<br><br>The NOEL for oncogenic effects was 1 ppm. |
| Reference: | Sellakumar, A. R. et al. (1987). J. National Cancer Soc., |

| | |
|---|---|
| | 79:285-289. |
| Reliability: | High because a scientifically defensible or guideline method was used. |
| **Type:** | **18-22 Month Carcinogenicity Test** |
| Species/Strain: | Mice/Hsd:ICR(BR) |
| Sex/Number: | Female/30-50 per group |
| Exposure Period: | 18-22 months |
| Frequency of Treatment: | Dermal (2-stage): single application followed 2 weeks later by 3 times/week of phorbol myristate acetate (PMA)<br>Dermal (repeated application): 3 times/week<br>Subcutaneous injection: 1 time/week |
| Exposure Levels: | Dermal (2-stage): 3.0 mg/administration<br>Dermal (repeated application): 1.5, 3.0 mg/administration<br>Subcutaneous injection: 2.0 mg/administration |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. |

DCAC was tested by repeated skin application, in two-stage carcinogenesis with PMA as promoter, and by repeated subcutaneous injection. Dimethylcarbamyl chloride was used as a positive control together with control groups.

Mice were 6-8 weeks of age at study start. Food and water were available *ad libitum*. All treatments were conducted in ventilated treatment hoods having an air flow of at least 100 linear ft/min. After treatment, mice were housed in these hoods for 2-3 hours before being returned to the animal rooms.

For the repeated skin application test, DCAC in 0.1 mL acetone was applied 3 times/week for the duration of the test in the intrascapular region. Mice for these tests were shaved at the beginning of the test and then as needed at regular intervals.

In the 2-stage test, a single application of DCAC was applied, followed 2 weeks later by 3 times/week application of PMA (phorbol myristate acetate) for the duration of the test.

For the subcutaneous test, DCAC in 0.05 mL trioctanoin was injected in the left flank once weekly.

Animals were weighed at 30-60-day intervals. Tumor

observations were made daily. Animals that became moribund or died during the treatment period or had large tumor masses were killed. All mice were necropsied, and routine sections were taken from the area of administration as well as lung, liver, kidney, spleen, colon, and urinary bladder. Any other tissues and organs that appeared clinically abnormal were also taken for histopathology.

GLP: Unknown

Test Substance: DCAC, purity > 99%

Results: DCAC showed marginally significant incidences of papillomas and carcinomas when tested as an initiator.

DCAC did not show skin tumorigenicity in the repeated skin application test.

The tumor incidence when DCAC was tested via subcutaneous injection, as well as when tested as an initiator, cannot be disregarded.

| Dose[a] | Median survival time (days) | Days to first tumor | No. of mice with tumor/no. of mice tested | No. and tumor types at site of administration |
|---|---|---|---|---|
| Skin application (2-stage) test | | | | |
| 3.0 | 465/640 | 365 | 5/50 | 3 pap, 2 squa ca |
| Repeated skin application | | | | |
| 3.0 | 520/660 | -- | 0/50 | 0 |
| 1.5 | 485/660 | -- | 0/30 | 0 |
| Subcutaneous injection test | | | | |
| 2.0 | 510/660 | -- | 4/50 | 1 sarcoma, 1 squa ca, 1 hemangioma, 1 pap |

[a] mg/administration
pap = papilloma
squa ca = squamous carcinoma

Reference: Van Duuren, B. L. et al. (1987). J. Am. Coll. Toxicol., 6(4):479-488.

Reliability: High because a scientifically defensible or guideline method was used.

**Additional References for Repeated Dose Toxicity:**

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Van Duuren, B. L. et al. (1983). Cancer Res., 43:159.

Rosca, S. et al. (1982). Rev. Med. (Tirgu-Mures, Rom), 28(2):151-154 (HSDB/5229 and CA100:152211).

Szoverfi, A. B. et al. (1983). Rev. Med. (Tirgu-Mures, Rom), 29(1-2):77-80 (CA102:1550).

Data from these additional sources were not summarized because the focus of the study was immunological responses.

Khan, M. F. et al. (1995). Tox. Appl. Pharm., 134, 155-160.

Khan, M. F. et al. (1995). Toxicologist, 15(1):228 (also citedin TSCA Fiche OTS0557868).

Khan, M. et al. (1997). Immunopharm. Immunotox., 19(2):265-277 (BIOSIS/1997:266879).

**5.3 Developmental Toxicity:** No Data.

**5.4 Reproductive Toxicity:**

| | |
|---|---|
| Species/Strain: | Rats/Sprague-Dawley |
| Sex/Number: | Male/50 per DCAC group or 98 in the control group |
| Route of Administration: | Inhalation |
| Exposure Period: | 30 days |
| Frequency of Treatment: | 6 hours/day, 5 days/week |
| Exposure Levels: | 0, 0.5, 1.0, 2.0 ppm |
| Method: | A 30-day inhalation study was conducted in male rats (see Section 5.2 for details on the study design). Complete necropsies were performed at study termination. Testes were examined microscopically. |
| GLP: | Unknown |
| Test Substance: | DCAC, purity > 95% |
| Results: | No compound-related effects on the testes were observed. Nasal tumors were present at 2 ppm. Two of 50 animals had squamous cell carcinoma or mixed cell carcinoma of nasal mucosa. These animals died 701 and 887 days after the initial exposure. No tumors were observed in the controls. |

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| Reference: | Sellakumar, A. R. et al. (1987). J. National Cancer Soc., 79:285-289. |
| Reliability: | Medium because a suboptimal study design was used. |

**Additional References for Reproductive Toxicity:** None Found.

## 5.5 Genetic Toxicity

| | |
|---|---|
| **Type:** | **Prophage-induction assay**<br>***In vitro* Bacterial Reverse Mutation Assay**<br>**Molecular analysis of *Salmonella* revertants** |
| Tester Strain: | Prophage-induction assay: *E. coli* B/r<br>*Salmonella* mutagenicity assay: T100<br>Molecular analysis of *Salmonella* revertants: TA100 |
| Exogenous Metabolic Activation: | With and without Aroclor®-induced rat liver S9 |
| Exposure Concentrations: | Prophage-induction assay (-S9): 0-5 mg/mL<br>Prophage-induction assay (+S9): 0-10 mg/mL<br>*Salmonella* mutagenicity assay (-S9): 0-600 ppm<br>*Salmonella* mutagenicity assay (-S9): 0-700 ppm |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>Prophage-induction assay:<br>The lambda lysogen $WP2_s$(lambda) was derived from *E. coli* B/r. The indicator strain was TH-008 (Streptomycin$^r$). The assay was performed as described in DeMarini and Brooks, 1992 (DeMarini, D. M. and H. G. Brooks (1992). Environ. Mol. Mutagen., 19:98-111). Test substances were evaluated up to a maximum concentration of 10% (v/v).<br>2-Nitrofluorene (2-NF) was used as the positive control for the trials without metabolic activation and 2-aminoanthracene (2-AA) was used as the positive control for the trials with metabolic activation.<br><br>The first well in a dilution series of a 96-well microtiter plate received supplemented minimal medium and either test compound or medium control. The remaining wells received medium, and 2-fold serial dilutions of the test compound or controls were made down the columns of each plate. Each well was inoculated with a resuspended log-phase culture of $WP2_s$(lambda) and medium or S9 mix. After incubation overnight at 37°C, the wells were scored for turbidity. Turbid wells indicated cell growth and clear wells indicated |

cytotoxicity and/or inhibition of cell growth. The concentration of lambda bacteriophage was determined by sampling at least the first 5 turbid wells adjacent to a clear well. A sample from a well was diluted, plated onto indicator cells, and incubated overnight at 37°C. Plaque-forming units (PFU or plaques) were counted by hand. The dilution tubes were sampled and diluted in duplicate and all experiments were performed at least twice.

*Salmonella* mutagenicity assay (bag vaporization method): The vaporization technique was performed as described by Hughes et al., 1987 (Hughes, T. J. et al. (1987). Environ. Mutagen., 9:421-441) with the following modifications. Top agar containing an overnight culture of strain TA100 (± S9 mix) was poured onto minimal medium in a glass petri dish. After the top agar had hardened, the bottom and top parts of the petri dish were placed against each other, and the assembly was inserted into a Tedlar® bag of known volume (600-800 mL) with the inverted top of the dish directly under the septum of the bag. The bag was then sealed, and various amounts of test substance were injected through a septum on the bag into the inverted top of the petri dish. The bag was placed in a 37°C incubator for 24 hours. The bag was then opened, the 2 halves of the petri were reassembled, and the inverted plate was placed back into the incubator for an additional 48 hours. Colonies were counted by an automatic colony counter. Each petri plate was in a separate bag, and 2 plates were exposed at each concentration of chemical tested. All experiments were performed at least twice. Sodium azide (without metabolic activation) and 2-AA (with metabolic activation) were used as positive controls. A reproducible, 2-fold increase in revertants/plate relative to the background was considered a positive response.

Molecular analysis of *Salmonella* revertants: Approximately 1200 revertants of TA100 (at least 200 from each treatment group) were analyzed using the colony probe hybridization procedure developed by Cebula and Koch, 1990 (Cebula, T. A. and W. H. Koch (1990). Mutat. Res., 229:79-87) with the modification of Shelton et al., 1994 (Shelton, M. L. et al. (1994). Mutat. Res., 323:35-39). These were compared to approximately 600 background revertants from laboratory historical control. The selected revertants were streaked onto minimal medium supplemented with biotin and incubated for 2 days at 37°C in order to purify each revertant clone from any nonrevertant cells that might

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| | have been transferred from the background lawn. Two independent hybridizations were performed with each probe on each revertant in order to confirm the revertant genotype. Six revertant strains of known genotype were used as positive controls. Mutation spectra were compared by Chi-square analysis using the Stat-Sak program. |
| GLP: | Unknown |
| Test Substance: | DCAC, purity 99% |
| Results: | Positive |
| Remarks: | In the Microscreen prophage-induction assay, DCAC was positive in experiment 2, producing a 3-fold increased in PFU/plate relative to the background; however, a 3-fold increase or greater was not achieved in experiment 1.<br><br>DCAC was positive in TA100 without metabolic activation at 600 ppm and negative in TA100 with metabolic activation when tested at 0-700 ppm.<br><br>Chi-square analysis indicated that DCAC mutation spectra were significantly different from the background mutation spectra. DCAC induced primarily GC to AT transitions. |
| Reference: | DeMarini, D. M. et al. (1994). Mutagenesis, 9(5):429-437. |
| Reliability: | High because a scientifically defensible or guideline method was used. |
| **Type:** | ***In vitro* Bacterial Reverse Mutation Test** |
| Tester Strain: | *Salmonella typhimurium* strains TA98 and TA100 |
| Exogenous Metabolic Activation: | With and without 10 and 30% Aroclor®-induced rat and hamster liver S9 |
| Exposure Concentrations: | 0, 3, 33, 100, 333, 666, 1000, 1666, 3333, and 6666 µg/plate<br>Comment: Not all exposure concentrations were tested with all tester strains under all test conditions. |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>The preincubation method originally described by Haworth et al., 1983 (Haworth, S. et al. (1983). Environ. Mutagen., 6(Suppl. 1):3-142) was used with some modifications. The test substance, overnight culture of *Salmonella*, and S9 mix or buffer were incubated at 37°C, without shaking for 20 minutes. Test substances known or suspected to be volatile were incubated in capped tubes. The top agar was added and the contents of the tubes were mixed and poured onto the surface of petri dishes containing medium. |

Histidine-independent (his+) colonies arising on these plates were counted following 2 days incubation at 37°C. Plates were machine counted (New Brunswick, Artek). At the discretion of the investigator, plates with low numbers of colonies, containing precipitated test substance, or having excessively-reduced contrast because of chemical color, were counted by hand.

The initial test of a test substance was without activation and with 10% S9. If a positive result was obtained, the positive trial(s) was repeated. If the trials were negative the test substance was retested without S9 and with 30% S9. If all trials were negative, no further testing was performed.

A test substance was designated nonmutagenic only after it had been tested in strains TA97, TA98, TA100, TA1535, and TA1537, without exogenous activation, and with 10% and 30% rat and hamster S9.

DCAC was run initially in a toxicity assay using TA100 or the system developed by Waleh et al., 1982 (Waleh, N. S. et al. (1982). Mutat. Res., 97:247-256). Toxic concentrations were defined as those that produced a decrease in the number of his+ colonies, or a clearing in the density of the background lawn, or both.

The test substance was initially tested in the preincubation test at half-log dose intervals up to a dose that elicited toxicity, or to a dose immediately below one that was toxic in the preliminary toxicity procedure. Subsequent trials occasionally used narrower dose increments, and may not have included doses in the toxic range. At least 5 doses of the test substance were tested in triplicate, and repeat experiments were performed at least 1 week following the initial trial.

Concurrent solvent (acetone) and positive controls were run with each trial. The positive controls in the absence of exogenous metabolic activation were sodium azide (TA100) and 4-nitro-o-phenylenediamine (TA98). The positive control for exogenous metabolic activation with all strains was 2-aminoanthracene.

The test substance was considered mutagenic or weakly mutagenic if it produced a reproducible, dose-related response over the solvent control, under a single metabolic

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|---|---|
| | activation condition, in replicate trials. The test substance was considered questionable if the results of individual trials were not reproducible, if increases in his+ revertants did not meet the criteria for a weakly positive response, or if only single doses produced increases in his+ revertants in repeat trials. The test substance was judged nonmutagenic if it did not meet the criteria for a mutagenic or questionable response. |
| GLP: | Unknown |
| Test Substance: | DCAC, purity ~ 97% |
| Results: | Positive |
| Remarks: | DCAC was positive in TA100 without metabolic activation (doses 3-333 µg/plate), negative in TA100 with metabolic activation (rat and hamster) (doses 100-6666 µg/plate), negative in TA98 with metabolic activation (doses 100-6666 µg/plate) (rat and hamster), and negative in TA98 without metabolic activation (doses 3-3333 µg/plate). |
| Reference: | Zeiger, E. et al. (1992). Environ. Mol. Mutagen., 19(Suppl. 21):2-141. |
| Reliability: | High because a scientifically defensible or guideline method was used. |

**Additional References for *In vitro* Bacterial Reverse Mutation Assay:** None Found.

**Type:** ***In vitro* Clastogenicity Studies:** No Data.

**Type:** ***In vivo* Mouse Micronucleus Assay:** No Data.

APPENDIX B

# ROBUST SUMMARY FOR DICHLOROACETIC ACID

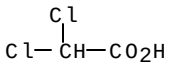
Existing published and unpublished data were collected and scientifically evaluated to determine the best possible study or studies to be summarized for each required endpoint. In the spirit of this voluntary program, other data of equal or lesser quality are not summarized, but are listed as related references at the end of each appropriate section, with a statement to reflect the reason why these studies were not summarized.

## 1.0 Substance Information

**CAS Number:** 79-43-6

**Chemical Name:** Dichloroacetic acid

**Structural Formula:**



**Other Names:**
DCA
DCA (acid)
Dichloracetic acid
Dichlorethanoic acid
Dichloroethanoic acid

**Exposure Limits:** ACGIH TLV: 0.5 ppm; skin; A3

## 2.0 Physical/Chemical Properties

### 2.1 Melting Point

| | |
|---|---|
| Value: | 13.5ºC |
| Decomposition: | No Data |
| Sublimation: | No Data |
| Pressure: | No Data |
| Method: | No Data |
| GLP: | Unknown |
| Reference: | Lide, D. R. (1995-1996). CRC Handbook of Chemistry, 5th ed., pp. 3-7, CRC Press, Inc., Boca Raton, FL (HSDB/6894). |
| Reliability: | Not assignable because limited study information was available. |

**Additional References for Melting Point:**

US EPA (2003). Toxicological Review of Dichloroacetic Acid (August), Document No. EPA 635/R-03/007.

Anon. (1995). IARC Monograph Eval. Carcinogen. Risks Hum., 63:271-290.

## 2.2 Boiling Point

| | |
|---|---|
| Value: | 193-194ºC |
| Decomposition: | No Data |
| Pressure: | No Data |
| Method: | No Data |
| GLP: | Unknown |
| Reference: | Budavari, S. (1981). The Merck Index – Encyclopedia of Chemicals, Drugs, and Biologicals, p. 481, Merck and Co., Inc., Rahway, NJ (HSDB/6894). |
| Reliability: | Not assignable because limited study information was available. |

**Additional References for Boiling Point:**

US EPA (2003). Toxicological Review of Dichloroacetic Acid (August), Document No. EPA 635/R-03/007.

Anon. (1995). IARC Monograph Eval. Carcinogen. Risks Hum., 63:271-290.

## 2.3 Density

| | |
|---|---|
| Value: | Density: 1.5724 g/mL; Specific gravity: 1.563 |
| Temperature: | Density: 13ºC; Specific gravity: 20/4ºC |
| Method: | No Data |
| GLP: | Unknown |
| Results: | No additional data. |
| Reference: | US EPA (2003). Toxicological Review of Dichloroacetic Acid (August), Document No. EPA 635/R-03/007. |
| Reliability: | Not assignable because limited study information was available. |

**Additional References for Density:**

Budavari, S. (1981). The Merck Index – Encyclopedia of Chemicals, Drugs, and Biologicals, p. 481, Merck and Co., Inc., Rahway, NJ (HSDB/6894).

Anon. (1995). IARC Monograph Eval. Carcinogen. Risks Hum., 63:271-290.

## 2.4 Vapor Pressure

| | |
|---|---|
| Value: | 0.179 mm Hg |
| Temperature: | 25$^{o}$C |
| Decomposition: | No Data |

| | |
|---|---|
| Method: | No Data |
| GLP: | Unknown |
| Reference: | Daubert, T. E. and R. P. Danner (1989). Physical and Thermodynamic Properties of Pure Chemicals Data Compilation, Taylor and Francis, Washington, DC (HSDB/6894). |
| Reliability: | Not assignable because limited study information was available. |

**Additional References for Vapor Pressure:**

US EPA (2003). Toxicological Review of Dichloroacetic Acid (August), Document No. EPA 635/R-03/007.

Hoechst Chemicals (1990). Chemical Information Sheet: Dichloroacetic acid, Dallas, TX (cited in Anon. (1995). IARC Monograph Eval. Carcinogen. Risks Hum., 63:271-290).

## 2.5 Partition Coefficient (log Kow)

| | |
|---|---|
| Value: | 0.52 |
| Temperature: | 25$^{o}$C |
| Method: | Modeled. KOWWIN, v1.67, module of EPIWIN v3.11 (Syracuse Research Corporation). KOWWIN uses "fragment constant" methodologies to predict log P. In a "fragment constant" method, a structure is divided into fragments (atom or larger functional groups) and coefficient values of each fragment or group are summed together to yield the log P estimate. |
| GLP: | Not Applicable |
| Reference: | Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92. |
| Reliability: | Estimated value based on accepted model. |

| | |
|---|---|
| Value: | 0.92 |
| Temperature: | No Data |
| Method: | Measured |
| GLP: | Unknown |
| Reference: | Hansch, C. et al. (1995). Exploring QSAR – Hydrophobic, Electronic, and Steric Constants, p. 4, American Chemical Society, Washington, DC (HSDB/6894). |
| Reliability: | Not assignable because limited study information was available. |

**Additional References for Partition Coefficient (log Kow):** None Found.

## 2.6 Water Solubility

| | |
|---|---|
| Value: | 1E+006 mg/L |
| Temperature: | 20°C |
| pH/pKa: | Based on a measured pKa of 1.26 (Maruthamuthu and Huie, 1995), dichloroacetic acid is expected to mainly exist as an anion at environmental pH values (SRC, n.d.). |
| Method: | Yalkowsky and Dannenfelser, 1992.<br><br>pKa – SPARC on-line calculator, University of Georgia. |
| GLP: | Not Applicable |
| Reference: | Yalkowsky, S. H. and R. M. Dannenfelser (1992). The AQUASOL DATABASE of Aqueous Solubility, Ver 5, Univ. AZ, College of Pharmacy, Tuscon, AZ.<br><br>Solubility - Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106.<br><br>pKa<br>Maruthamuthu, P. and R. E. Huie (1995). Chemosphere, 30:2199-207 (HSDB/6894).<br><br>SRC (Syracuse Research Corporation) (n.d.). (HSDB/6894). |
| Reliability: | Solubility: Data from handbook.<br>pKa: Estimated values based on accepted models. |

**Additional References for Water Solubility:**

US EPA (2003). Toxicological Review of Dichloroacetic Acid (August), Document No. EPA 635/R-03/007.

Budavari, S. (1981). The Merck Index – Encyclopedia of Chemicals, Drugs, and Biologicals, p. 481, Merck and Co., Inc., Rahway, NJ (HSDB/6894).

Gerhatz, W. (1985 to present). Ullmann's Encyclopedia of Industrial Chemistry, 5th ed., Vol. A1, p. VA6 543, VCH Publishers, Deerfield Beach, FL (HSDB/6894).

Hoechst Chemicals (1990). Chemical Information Sheet: Dichloroacetic acid, Dallas, TX (cited in Anon. (1995). IARC Monograph Eval. Carcinogen. Risks Hum., 63:271-290).

**2.7 Flash Point:** No Data.

**2.8 Flammability:** No Data.

## 3.0 Environmental Fate

### 3.1 Photodegradation

| | |
|---|---|
| Concentration: | Not Applicable |
| Temperature: | Not Applicable |
| Direct Photolysis: | Iron complexes of DCA undergo photolytic decomposition. |
| Indirect Photolysis: | Estimated atmospheric half-life of 22 days due oxidation by hydroxyl radicals. |
| Breakdown Products: | Not Applicable |
| Method: | According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere (Bidleman, 1988), dichloroacetic acid, which has a measured vapor pressure of 0.179 mm Hg at 25ºC (Daubert and Danner, 1989; SRC, n.d.), from experimentally-derived coefficients, will exist solely as a vapor in the ambient atmosphere. Vapor-phase dichloroacetic acid is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals (SRC, n.d.). The rate constant for the gas-phase reaction of dichloroacetic acid with photochemically produced hydroxyl radicals has been estimated as $7.3 \times 10^{-12}$ $cm^3$/molecule-sec at 25ºC, which corresponds to a half-life of 22 days at an atmospheric concentration of $5 \times 10^5$ hydroxyl radicals/$cm^3$ (Meylan and Howard, 1993; SRC, n.d.).<br><br>Dichloroacetic acid may be photolyzed in aqueous solutions (Franke et al., 1994). Aqueous solutions of ferric ions and dichloroacetic acid were photolyzed by light with wavelengths > 300 nm; dichloroacetic acid was photolyzed at a rate of $2 \times 10^{-7}$ eins/sec-mL (Maruthamuthu and Huie, 1995). |
| GLP: | Not Applicable |
| Reference: | Bidleman, T. F. (1988). Environ. Sci. Technol., 22:361-367 (HSDB/6894).<br><br>Daubert, T. E. and R. P. Danner (1989). Data Compilation, Tables of Properties of Pure Compounds, Design Inst. Phys. Prop. Data, Am. Inst. Phys. Prop. Data NY, NY(HSDB/6894). |

Franke, C. et al. (1994). Chemosphere, 29:1501-1514 (HSDB/6894).

Meylan, W. M. and P. H. Howard (1993). Chemosphere, 26:2293-99 (HSDB/6894).

Maruthamuthu, P. and R. E. Huie (1995). Chemosphere, 30:2199-207 (HSDB/6894).

SRC (n.d.). Syracuse Research Corporation (HSDB/6894).

Reliability: Estimated value based on accepted model.

**Additional Reference for Photodegradation:**

Data from this additional source supports the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Pupo Nogueira, R. F. and J. R. Guimarares (2000). Water Res., 34(3):895-901 (BIOSIS/2000:112673).

## 3.2 Stability in Water

Concentration: No Data
Half-life: No Data
% Hydrolyzed: No Data
Method: Based on a recommended classification scheme (Lyman et al., 1990), an estimated Koc value of 75 (SRC, n.d.), determined from a measured log Kow (Hansch et al., 1995) and a recommended regression-derived equation (Lyman et al., 1990), indicates that dichloroacetic acid should not adsorb to suspended solids and sediment in water (SRC, n.d.). Under environmental pH conditions, dichloroacetic acid should exist mainly as the anion (SRC, n.d.), based on its pKa value (Maruthamuthu and Huie, 1995). Dichloroacetic acid is not expected to volatilize from water surfaces (Lyman et al., 1990; SRC, n.d.) given a measured Henry's Law constant of $3.52 \times 10^{-7}$ atm-$m^3$/mole (SRC, n.d.), developed using a fragment constant estimation method (Meylan and Howard, 1991). Dichloroacetic acid may be photolyzed in aqueous solutions (Franke et al., 1994). Carboxylic acids are generally resistant to hydrolysis in water (Lyman et al., 1990).
GLP: Not Applicable
Reference: Lyman, W. J. et al. (1990). Handbook of Chemical Property Estimation Methods, pp. 4-9, 5-4, 5-10, 15-1 to 15-29,

Amer. Chem. Soc., Washington, DC (HSDB/6894).

Hansch, C. et al (1995). Exploring QSAR, Hydrophobic, Electronic, and Steric Constants. p. 4, Amer. Chem. Soc., Washington, DC (HSDB/6894).

Maruthamuthu, P. and R. E. Huie (1995). Chemosphere, 30:2199-2207 (HSDB/6894).

Meylan, W. M. and P. H. Howard (1991). Environ. Toxicol. Chem., 10:1283-93 (HSDB/6894).

Franke, C. et al. (1994). Chemosphere, 29:1501-1514 (HSDB/6894).

SRC (n.d.). Syracuse Research Corporation (HSDB/6894).

Reliability: Estimated value based on accepted model.

**Additional References for Stability in Water:** None Found.

### 3.3 Transport (Fugacity):

Media: Air, Water, Soil, and Sediments

Distributions:

| Compartment | % of total distribution | ½ life hours (advection + reaction) |
|---|---|---|
| Air | 4.05 | 527 |
| Water | 38.8 | 360 |
| Soil | 57.2 | 720 |
| Sediment | 0.077 | 3240 |

Adsorption Coefficient: Koc = 3.41

Desorption: Not Applicable

Volatility: Henry's Law Constant = 3.52e-007 atm-$m^3$/mole (HENRYWIN database)

Method: Environmental Distribution - Mackay Level III fugacity model, in EPIWIN v3.11 (Syracuse Research Corporation). Emissions (1000 kg/hr) to air, water, and soil compartments.

Data Used:
SMILES: O=C(O)C(CL)CL
Vapor Pressure: 0.179 mm Hg (experimental)
Log Kow: 0.92

Henry's Law Constant - HENRYWIN v3.10 module of EPIWIN v3.11 (Syracuse Research Corporation). Henry's Law Constant (HLC) is estimated by two separate methods

that yield two separate estimates. The first method is the bond contribution method and the second is the group contribution method. The bond contribution method is able to estimate many more types of structures; however, the group method estimate is usually preferred (but not always) when all fragment values are available.

Log Koc – Calculated from log Kow by the Mackay Level III fugacity model incorporated into EPIWIN v3.11 (Syracuse Research Corporation).

GLP: Not Applicable

Reference: HENRYWIN –

Hine, J. and P. K. Mookerjee (1975). J. Org. Chem., 40(3):292-298.

Meylan, W. and P. H. Howard (1991). Environ. Toxicol. Chem., 10:1283-1293.

Fugacity - The methodology and programming for the Level III fugacity model incorporated into EPIWIN v3.11 (Syracuse Research Corporation) were developed by Dr. Donald MacKay and coworkers and are detailed in:

Mackay, D. (1991). Multimedia Environmental Models: The Fugacity Approach, pp. 67-183, Lewis Publishers, CRC Press.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.

Reliability: Estimated values based on accepted models.

**Additional References for Transport (Fugacity):** None Found.

## 3.4 Biodegradation

Value: At 100 mg/L, dichloroacetic acid was 97% degraded in 14 days using an activated sludge inoculum in a MITI test format (Chemicals Inspection and Testing Institute, 1992). The biodegradability of dichloroacetic acid, at 10 ppm, was measured in both river water and seawater using the cultivation method; 14 and 8% degradation was reported for river water and seawater, respectively, after 3 days incubation (Kondo et al., 1988). Based on these results, this

| | |
|---|---|
| | compound was determined to be difficult to degrade (Kondo et al., 1988). 0, 27, and 68% of the theoretical BOD in a BOD test was reached in 2, 5, and 10 days, respectively, following inoculation with sewage (Dias and Alexander, 1971). Dichloroacetic acid was not biodegraded during a 5-day BOD test using a sewage inoculum (Heukelekian and Rand, 1955). Dichloroacetic acid at 20 mg/L was >95% degraded in a 20-day BOD test; in a 2nd screening test, this compound was 83% degraded after 30 days (Popp, 1985). Pure culture experiments show that aerobic degradation occurs via dehalogenation (Hirsch and Alexander, 1990). |
| Breakdown Products: | No Data |
| Method: | Varied (MITI Test, BOD Test, cultivation method) |
| GLP: | Unknown |
| Reference: | Kondo, M. et al. (1988). Eisei Kagaku, 34:188-195 (HSDB/6894).<br><br>Dias, F. F. and M. Alexander (1971). Appl. Microbiol., 22:1114-1118 (HSDB/6894).<br><br>Heukelekian, H. and M. C. Rand (1955). J. Water Pollut. Contr. Assoc., 29:1040-1053 (HSDB/6894).<br><br>Popp, K. H. (1985). GWP, Gaswasserfach: Wasser/Abwasser, 126:286-292 (HSDB/6894).<br><br>Chemicals Inspection and Testing Institute (1992). Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. Japan Chemical Industry Ecology - Toxicology and Information Center, ISBN 4-89074-101-1 (HSDB/6894).<br><br>Hirsch, P. and M. Alexander (1990). Canadian J. Microbiol., 6:241-249 (HSDB/6894). |
| Reliability: | High because a scientifically defensible or guideline method was used. |

**Additional References for Biodegradation:**

Data from these additional source support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Ellis, D. A. et al. (2001). Chemosphere, 42:309.

Heinze, U. and H. J. Rehm (1993). Appl. Microbiol. Biotechnol., 40(1):158-164 (BIOSIS/1994:16373).

Meusel, M. and H. J. Rehm (1993). Appl. Microbiol. Biotechnol., 40(1):165-171 (BIOSIS/1994:16372).

Data from this additional source were not summarized because portions of the fiche were unreadable.

Dow Chemical Co. (1965). "The Pollution Evaluation of Compounds with "Red Flag" Designations" (October 1) (TSCA Fiche OTS0530112).

### 3.5 Bioconcentration

| | |
|---|---|
| Value: | BCF = 3. According to a classification scheme (Franke et al., 1994), this BCF value suggests that bioconcentration in aquatic organisms is low (SRC, n.d.). |
| Method: | An estimated BCF value was calculated (SRC, n.d.) using a measured log Kow of 0.92 (Hansch et al., 1995) and a recommended regression-derived equation (Lyman et al., 1990). |
| GLP: | Not Applicable |
| Reference: | Hansch, C. et al. (1995). Exploring QSAR, Hydrophobic, Electronic, and Steric Constants. p. 4, Amer. Chem. Soc., Washington, DC (HSDB/6894).<br><br>Lyman, W. J. et al. (1990). Handbook of Chemical Property Estimation Methods, pp. 5-4, 5-10, Amer. Chem. Soc., Washington, DC (HSDB/6894).<br><br>Franke, C. et al. (1994). Chemosphere, 29:1501-1514 (HSDB/6894).<br><br>SRC (n.d.). Syracuse Research Corporation (HSDB/6894). |
| Reliability: | Estimated value based on accepted model. |

**Additional References for Bioconcentration:** None Found.

## 4.0 Ecotoxicity

### 4.1 Acute Toxicity to Fish

| | |
|---|---|
| **Type:** | **24-hour $LC_{50}$** |
| Species: | Zebrafish |
| Value: | Approx. 100 mg/L |
| Method: | No specific test guideline was reported; however, a |

| | |
|---|---|
| | scientifically defensible approach was used to conduct the study.<br><br>Zebrafish embryos were generated by natural pairwise mating as described by Westerfield, M. (1993). The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish, The University of Oregon Press, Eugene, OR. Day 1 embryos were distributed into a 96-well microplate, 1-3 embryos per well.<br><br>Ten 24 hpf embryos were exposed to varying concentrations of the test substance (in general from 100 nM to 100 µM in the presence of 0.1% DMSO) continuously for 5 days. Exact concentrations were not reported. The test substance was renewed daily, and pH and ammonia concentrations were monitored. The series of dilutions was repeated 4 times and the standard deviation was calculated for each treatment. $LC_{50}$ values were determined. |
| GLP: | Unknown |
| Test Substance: | DCA, purity not reported |
| Results: | No additional results for the lethality test were reported. |
| Reference: | Parng, C. et al. (2002). Assay Drug Develop. Technol., 1(1):41-48. |
| Reliability: | Medium because a suboptimal study design (nominal concentrations only) was used. |
| **Type:** | **96-hour $LC_{50}$** |
| Species: | Fish |
| Value: | 23,528 mg/L (using log Kow of 0.52) |
| Method: | Modeled |
| GLP: | Not Applicable |
| Test Substance: | DCA |
| Results: | No additional data. |
| Reference: | Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication). |
| Reliability: | Estimated value based on accepted model. |

**Additional References for Acute Toxicity to Fish:**

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were

not substantially additive to the database.

Applegate, V. C. et al. (1957). Spec. ScI. Rep. – Fish No. 207, p. 157, Fish Wildl. Serv., USD1, Washington, DC.

Loeb, H. A. and W. H. Kelly (1963). U.S. Fish & Wild. Serv. Spec. Sci. Rep., Fish No. 471, USD1, p. 124, Washington, DC.

Data from this additional source were not summarized because portions of the fiche were unreadable.

Dow Chemical Co. (1965). "The Pollution Evaluation of Compounds with "Red Flag" Designations" (October 1) (TSCA Fiche OTS0530112).

## 4.2 Acute Toxicity to Invertebrates

| | |
|---|---|
| **Type:** | **96-hour $LC_{50}$** |
| Species: | Harpacticoid copepod, *Nitocra spinipes* |
| Value: | 23.0 mg/L (95% confidence intervals, 21-25 mg/L) |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. |

The test was conducted under static conditions, in which the test solutions were not renewed during the 96-hour test period. No aeration of the test solutions was performed. Animals were not fed during the test.

The test was preceded by at least 1 pilot test under static conditions, in which the mortality interval was determined. In the main test, at least 6 concentrations and a control were used. Exact concentrations were not reported. No analyses for actual test substance in the test solutions were conducted. No attempt was made to adjust the pH.

2x10 *N. spinipes* were exposed to each test concentration. The *N. spinipes* were harvested from 3-6 week old cultures. They were tested in test tubes containing 10 mL natural brackish water, which was pumped from the Tvaren Bay in the Baltic Sea and was filtered through a folded paper filter. The following characteristics of the water were close to constant throughout the experiment: salinity of 7 o/oo, alkalinity of 1.5 meq/L, and pH of 7.8. The concentration of dissolved oxygen in the test water was measured at the end of the exposure period, and 5 mg/L was considered a satisfactory minimum level. The water was thermostated to

| | |
|---|---|
| | 21±1ºC and held constant in a thermoregulated room.<br><br>Mortality was recorded after 96 hours under a low power microscope with strong illumination. $LC_{50}$ values were determined by the graphical method described in Litchfield, J. T. and F. Wilcoxon (1949). J. Pharmacol., 96:99. |
| GLP: | Unknown |
| Test Substance: | DCA, purity 95% |
| Results: | No additional results were reported. |
| Reference: | Linden, E. et al. (1979). Chemosphere, 8(11-12):843-851. |
| Reliability: | Medium because a suboptimal study design (nominal concentrations only) was used. |

| | |
|---|---|
| **Type:** | **48-hour $EC_{50}$** |
| Species: | Daphnid |
| Value: | 22,761 mg/L (using log Kow of 0.52) |
| Method: | Modeled |
| GLP: | Not Applicable |
| Test Substance: | DCA |
| Results: | No additional data. |
| Reference: | Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication). |
| Reliability: | Estimated value based on accepted model. |

**Additional Reference for Acute Toxicity to Invertebrates:**

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Trenel, J. and R. Kuhn (1982). Umweltforschungsplan des Bundesministers des Innern, "Bewertung Wassergefahrdender Stoffe im Hinblick auf Lagerung, Umschlag, und Transport" (AQUIRE/AQ-0204401; AG-0204402).

## 4.3 Acute Toxicity to Aquatic Plants

| | |
|---|---|
| **Type:** | **14-Day $EC_{50}$** |
| Species: | *Myriophyllum spicatum*, *M. sibiricum*, and *Lemna gibba* |
| Value: | See table below in results for $EC_{50}$ values. |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the |

study.

*M. spicatum* and *M. sibiricum* were introduced into outdoor microcosms by transferring the plants to plastic "cone-tainers" filled with the same sifted soil used in the microcosm sediment trays. The tubes were placed into planting trays and soaked overnight in an irrigation pond to allow the soil to settle. The plants were then cut to 5 cm apical shoot lengths, with roots removed, and soaked in irrigation pond water to remove the media in which they were cultured. Every microcosm was supplied with 8 plants of each species evenly spaced in each tray. The plants were acclimatized for 1 day prior to the introduction of DCA.

Plants were sampled 1 day prior to dosing with DCA, and 4, 7, 14, and 21 days post-treatment. Somatic endpoints of growth (plant length), biomass (wet mass and dry mass), root number (primary roots from the plant stem), primary root lengths (total and longest), and the number of nodes were evaluated.

*L. gibba* was introduced into the microcosms immediately after DCA introduction for a 14-day exposure period. The plants were transferred from the laboratory colony to the microcosms where they were contained in floating wooden cages. Three plants with 4 fronds each were introduced into each of the 3 sections of the wooden cages. Frond number, plant number, wet and dry mass, and 14-day growth rates for both fronds and plants were determined.

DCA was added to the microcosms at exposure concentrations of 0, 3, 10, 30, and 100 mg/L. Each treatment was assigned to 3 separate microcosms. The DCA was dissolved in redistilled deionized water. The resulting solutions were neutralized to pH 7-8.5 with sodium hydroxide. Immediately prior to treatment, the waterflow into each microcosm from the main irrigation pond was terminated, creating a closed system. Water samples for DCA analysis and for routine water chemistry determinations were taken periodically throughout the study. The water chemistry determinations included temperature, dissolved oxygen, water hardness, alkalinity, and pH. Measurements of photosynthetically active radiation were taken at regular intervals during the course of the study. DCA analyses were performed by ion chromatography in a method described by Ellis, D. et al. (2001). Chemosphere,

42:309-318.

*Myriophyllum* sp. response data were analyzed using General Linear Models of SAS 8.0. The effect of DCA concentration on each endpoint at specific time points was evaluated in a one-way analysis of variance (ANOVA). Any analysis that did not meet normality assumptions were natural ln or square root transformed. Any data that did not meet normality assumptions after transformation were compared with the Kruskis-Wallis one-way ANOVA and Dunnett's Test. The data at each time point was also evaluated using non-linear regression techniques.

*L. gibba* data were evaluated in a similar fashion. The average of the 3 subsections from the holding trays were averaged and the means analyzed in a one-way ANOVA in SAS 8.0. Data that did not meet normality assumptions were natural ln transformed. The data was then evaluated using non-linear regression techniques.

GLP: Unknown

Test Substance: DCA, purity 99+%

Results: 14-Day $EC_{50}$ values as calculated using linear and non-linear techniques are presented in the table below.

| **Species** | **Endpoint** | **$EC_{50}$** |
|---|---|---|
| *M. spicatum* | Plant Length (cm) | 194.7 |
| *M. spicatum* | Root number | 135.9 |
| *M. spicatum* | Total root length (cm) | 114.4 |
| *M. spicatum* | Longest root length (cm) | 264.3 |
| *M. spicatum* | Node number | 249.9 |
| *M. spicatum* | Wet mass (mg) | 169.7 |
| | | |
| *M. sibiricum* | Plant Length (cm) | 111.0 |
| *M. sibiricum* | Root number | 186.6 |
| *M. sibiricum* | Total root length (cm) | 94.9 |
| *M. sibiricum* | Longest root length (cm) | 166.6 |
| *M. sibiricum* | Node number | 171.0 |
| *M. sibiricum* | Wet mass (mg) | 67.6 |
| *M. sibiricum* | Dry mass (mg) | 190.7 |
| | | |
| *L. gibba* | Frond number | 29.8 |
| *L. gibba* | Plant number | 29.8 |
| *L. gibba* | Wet mass (mg) | 36.4 |
| *L. gibba* | Dry mass (mg) | 41.2 |
| *L. gibba* | Frond growth rate | 158.2 |
| *L. gibba* | Plant growth rate | 181.5 |

Chemical and physical characteristics of the microcosms averaged over the 21-day study are presented in the table below.

| DCA (mg/L) | Temp (ºC) | pH | DOa (mg/L) | Alkalinity b | Hardness b | PARc |
|---|---|---|---|---|---|---|
| 0 | 19.8-23.9 | 7.8 | 9.4 | 175 | 323 | 332 |
| 3 | 19.6-24.0 | 7.8 | 9.0 | 178 | 335 | 301 |
| 10 | 19.6-24.3 | 7.6 | 8.5 | 173 | 336 | 341 |
| 30 | 19.7-23.6 | 7.6 | 9.1 | 172 | 337 | 311 |
| 100 | 19.5-24.1 | 7.4 | 7.6 | 159 | 340 | 336 |

a = DO is dissolved oxygen.
b = measured as mg/L of $CaCO_3$
c = PAR is photosynthetically active radiation. Measurements taken at a depth of 60 cm.

Reference: Hanson, M. L. et al. (2003). Ecotox. Environ. Saf., 55(1):46-63
Reliability: High because a scientifically defensible or guideline method was used.

**Type:** **96-hour $EC_{50}$**
Species: Green algae
Value: 13,820 mg/L (using log Kow of 0.52)
Method: Modeled
GLP: Not Applicable
Test Substance: DCA
Results: No additional data.
Reference: Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability: Estimated value based on accepted model.

**Additional Reference for Acute Toxicity to Aquatic Plants:**

Data from this additional source supports the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Trenel, J. and R. Kuhn (1982). Umweltforschungsplan des Bundesministers des Innern, "Bewertung Wassergefahrdender Stoffe im Hinblick auf Lagerung, Umschlag, und Transport."

## 5.0 Mammalian Toxicity

### 5.1 Acute Toxicity

| | |
|---|---|
| **Type:** | **Oral $LD_{50}$** |
| Species/Strain: | Rat/Paper indicated that the methods were basically the same as earlier studies by these authors. These studies used the Wistar or Sherman strains. |
| Value: | 2820 mg/kg |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>Four groups of 5 non-fasted male rats were given single oral dosages of DCA. Although the 4 dosages used were not given, they were in a geometrical series such as 1, 2, 4, 8 g/kg. The $LD_{50}$ was obtained graphically. |
| GLP: | No |
| Test Substance: | DCA, purity not reported |
| Results: | No additional data. |
| Reference: | Smyth, H. F., Jr. et al. (1951). Arch. Ind. Hyg. Occup. Med., 4:119.<br><br>Smyth, H. F., Jr. et al. (1949). J. Indust. Hyg. Toxicol., 31:60. |
| Reliability: | High because a scientifically defensible or guideline method was used. |

| | |
|---|---|
| **Type:** | **Oral $LD_{50}$** |
| Species/Strain: | Rats/Not specified<br>Mice/Albino |
| Value: | Rats: 4480 mg/kg (4290-4690 mg/kg)<br>Mice: 5520 mg/kg (3810-8000 mg/kg) |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>DCA was adjusted with NaOH to a range of pH between 6 and 7. Rats were fasted 18 hours prior to test substance administration. Ten animals per group were used. Mice received 3000, 3162, 4000, 5012, 5623, 6310, 7943, or 8913 mg/kg DCA. Rats received 2200, 2500, 2800, 3200, |

3600, 4000, 4400, or 4800 mg/kg DCA. The animals were observed for 6 days following treatment.

Dosage-mortality curves were constructed according to the methods of Bliss, C. I. (1935). Ann. Appl. Biol., 22:134 and according to the example described by Laug, E. P. et al. (1939). J. Ind. Hyg. Toxicol., 21:173.

GLP: No

Test Substance: DCA, purity not reported

Results: The animals receiving DCA quickly passed into a state of narcosis or seminarcosis, and within 36 hours either recovered or died without coming out of the narcosis. Mortality ratios are shown in the table below.

| Mice | | Rats | |
|---|---|---|---|
| Dose (mg/kg) | Mortality Ratio | Dose (mg/kg) | Mortality Ratio |
| 3000 | 2/10 | 2200 | 0/10 |
| 3162 | 1/10 | 2500 | 0/10 |
| 4000 | 2/10 | 2800 | 0/10 |
| 5012 | 3/10 | 3200 | 0/10 |
| 5623 | 4/10 | 3600 | 0/10 |
| 6310 | 7/10 | 4000 | 1/10 |
| 7943 | 8/10 | 4400 | 4/10 |
| 8913 | 9/10 | 4800 | 8/10 |

Reference: Woodard, G. et al. (1941). J. Ind. Hyg. Toxicol., 23:78-81.

Reliability: High because a scientifically defensible or guideline method was used.

**Additional References for Acute Oral Toxicity:**

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Traina, V. et al (1977). Ciba-Geigy Pharmaceuticals Unpublished Report No. 7-77, "CGS 7937A (Sodium dichloroacetate): Acute and subacute toxicity studies in mice, rats, and dogs" (cited in Katz, R. et al. (1981). Toxicol. Appl. Pharmacol., 57:273-287).

Yount, E. A. et al. (1982). J. Pharmacol. Exp. Ther., 222(2):501-508.

Evans, O. B. and P. W. Stacpoole (1982). Biochem. Pharmacol., 31:1295-1300 (cited in EPA (2003). EPA 635/R-03/007, "Toxicological Review of Dichloroacetic Acid in support of IRIS" (August)).

**Type: Inhalation**

| | |
|---|---|
| Species/Strain: | No Data |
| Exposure Time: | No Data |
| Value: | No Data |
| Method: | No Data |
| GLP: | No Data |
| Test Substance: | DCA |
| Results: | DCA has a very low vapor pressure and is not expected to volatilize from drinking water or contaminated environmental media to any appreciable extent. |
| Reference: | US EPA (2003). Toxicological Review of Dichloroacetic Acid (August), Document No. EPA 635/R-03/007. |
| Reliability: | Not applicable |

**Additional References for Acute Inhalation Toxicity:** None Found.

| | |
|---|---|
| **Type:** | **Dermal $LD_{50}$** |
| Species/Strain: | Rabbits/Albino |
| Exposure Time: | 24 hours |
| Value: | 0.51 mL/kg |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>Undiluted DCA was applied to the clipped skin of a rabbit trunk using a modification of the rubber cuff of the FDA (Draize et al. (1944). J. Pharmacol. Exper. Therap., 82:377). The dose was retained under a flexible film of rubber, vinyl plastic, or the like, selected to be impervious to the chemical. Dosages up to 20 mL/kg may have been used. The number of animals used per dosage was 5. The $LD_{50}$ was determined graphically. |
| GLP: | No |
| Test Substance: | DCA, purity not reported |
| Results: | No additional data. |
| Reference: | Smyth, H. F., Jr. et al. (1951). Arch. Ind. Hyg. Occup. Med., 4:119.<br><br>Smyth, H. F., Jr. and C. P. Carpenter (1944). J. Indust. Hyg. Toxicol., 26:269.<br><br>Smyth, H. F., Jr. and C. P. Carpenter (1948). J. Indust. Hyg. Toxicol., 30:63. |
| Reliability: | High because a scientifically defensible or guideline method was used. |

**Additional References for Acute Dermal Toxicity:** None Found.

| | |
|---|---|
| **Type:** | **Dermal Irritation** |
| Species/Strain: | Rabbits/Albino |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>An undiluted sample (0.01 mL) of DCA was applied to an area upon the clipped belly of a rabbit. The rabbit was observed after 24 hours, recording necrosis, edema, erythema, or congestion of capillaries. If the undiluted material gave evidence of strong primary irritation, it was applied in the form of a 1% solution in acetone to locate the least concentration causing irritation.<br><br>Hazard from primary irritation was expressed in numerical grades, based on the reactions of 5 rabbits, scored somewhat similarly to the method of Draize.<br><br>Grade 1 showed no reaction whatever from the undiluted sample.<br>Grade 2 showed an average reaction equivalent to a trace of capillary injection.<br>Grade 3 showed strong capillary injection.<br>Grade 4 showed slight erythema.<br>Grade 5 showed strong erythema, edema, or slight necrosis.<br>Grade 6 was used if a 10% acetone solution gave no reaction more severe than edema.<br>Grade 7 was used if a 1% acetone solution gave no reaction more severe than edema.<br>Grade 8 was used if a 0.1% acetone solution gave no reaction more severe than edema.<br>Grade 9 was used if a 0.01% acetone solution gave no reaction more severe than edema.<br>Grade 10 was used if a weaker solution was determined to give no reaction more severe than edema. |
| GLP: | No |
| Test Substance: | DCA, purity not reported |
| Results: | The primary skin irritation score for rabbits in the study was determined to be a 7. |
| Reference: | Smyth, H. F., Jr. et al. (1951). Arch. Ind. Hyg. Occup. Med., 4:119.<br><br>Smyth, H. F., Jr. and C. P. Carpenter (1944). J. Indust. Hyg. Toxicol., 26:269. |

| | |
|---|---|
| | Smyth, H. F., Jr. et al. (1949). J. Indust. Hyg. Toxicol., 31:60. |
| Reliability: | Medium because a suboptimal study design was used. |

| | |
|---|---|
| **Type:** | **Dermal Irritation** |
| Species/Strain: | Rabbits/Not specified |
| Method: | No specific test guideline was reported. Secondary source reports that 2 mg was tested. Test duration was 24 hours. |
| GLP: | Unknown |
| Test Substance: | DCA, purity not reported |
| Results: | Severe |
| Reference: | Anon. (1986). Prehled Prumysloe Toxikolgie; Organicke Latky, p. 571 (cited in Lewis, R. J. (2000). Sax's Dangerous Properties of Industrial Chemicals, 10th ed., p. 1178-1179, John Wiley and Sons, Inc., New York). |
| Reliability: | Not assignable because limited study information was available. |

**Additional References for Dermal Irritation:** None Found.

**Type:** **Dermal Sensitization:** No Data.

| | |
|---|---|
| **Type:** | **Eye Irritation** |
| Species/Strain: | Rabbits/Albino |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. |

Measured volumes of the undiluted DCA were placed on the center of the cornea of an albino rabbit which was shown previously to have uninjured eyes. After 24 hours, the eye was observed for gross evidence of injury and for corneal necrosis revealed by fluorescein stain. Volumes used were 0.001, 0.005, 0.02, 0.1, and 0.5 mL. The actual volume used was based on previous experience. Not all volumes were tested.

In some cases, an excess of a solution of the chemical in a non-irritating solvent, such as water or propylene glycol, was also used. The concentration used was selected from the series 40, 15, 5, 1, and 0.1%.

The scoring system was described in Carpenter, C. P. and H. F. Smyth, Jr. (1946). Am. J. Ophth., 29:1363. An injury grade of 10 was given when excess of a 1% solution gave an injury of 5.0 points. A score of 5.0 points was described as a

| | |
|---|---|
| | severe injury. |
| GLP: | No |
| Test Substance: | DCA, purity not reported |
| Results: | The eye injury in rabbits was given an injury grade of 10. |
| Reference: | Smyth, H. F., Jr. et al. (1951). Arch. Ind. Hyg. Occup. Med., 4:119.<br><br>Smyth, H. F., Jr. and C. P. Carpenter (1944). J. Indust. Hyg. Toxicol., 26:269. |
| Reliability: | High because a scientifically defensible or guideline method was used. |

**Additional References for Eye Irritation:** None Found.

## 5.2 Repeated Dose Toxicity

| | |
|---|---|
| **Type:** | **3-Month Oral Toxicity Study** |
| Species/Strain: | Rats/Crl: COBS CD (SD)BR<br>Dogs/Beagle |
| Sex/Number: | Rats: Male and female/10-15 per sex per group<br>Dogs: Male and female/3-4 per sex per group |
| Exposure Period: | 13 weeks |
| Frequency of Treatment: | Daily |
| Exposure Levels: | Rats: 0, 125, 500, 2000 mg/kg<br>Dogs: 0, 50, 75, 100 mg/kg |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>Rats were housed individually in wire-bottom cages in animal rooms that were kept at 72-76ºF, 40-65% humidity, and a 12-hour light cycle. Food and water were available *ad libitum*. At study initiation, mean group body weights of male and female rats ranged from 154 – 156 g (males) and 131 –133 g (females). The control and high dosage groups contained 15 rats/sex while the low and middle dosage groups contained 10 rats/sex. The additional 5 rats/sex in the control and high dose groups were assigned as "recovery animals" and were monitored for an additional 4 weeks following completion of the 13-week dosage period.<br><br>Dogs were housed individually in fixed cages in animal rooms that were kept at 67-73ºF, 40-65% relative humidity, and a 12-hour light cycle. Food and water were available *ad libitum*. At study initiation, mean group body weights of |

male and female dogs ranged from 6.7 – 10.4 kg (males) and 5.8 –7.4 kg (females). The control and high dosage groups contained 4 dogs/sex while the low and middle dosage groups contained 3 dogs/sex. The additional 1 dog/sex in the control and high dose groups were assigned as "recovery animals" and were monitored for an additional 5 weeks following completion of the 13-week dosage period.

Rats were administered the test solutions, once daily, by gavage for 13 consecutive weeks. Control rats received the aqueous vehicle. Dogs received daily oral doses in gelatin capsules. Control dogs received empty gelatin capsules.

For both species, the following endpoints were evaluated. Physical examinations were conducted initially and monthly thereafter. Ophthalmological exams were conducted initially and terminally for both species and dogs were also examined after study weeks 2 and 7. Behavioral examinations were conducted weekly. Body weights were recorded weekly and food consumption was recorded weekly for rats and daily for dogs. Daily observations for mortality, pharmacological and/or toxicological effects were also conducted.

Blood samples for hematological and biochemical determinations were collected at 0, 3, and 4 months. The animals were fasted overnight and blood was drawn from the tail vein or aortic artery (rats) and the jugular vein (dogs). Total erythrocyte and leukocyte counts were determined as well as differential leukocyte counts, hematocrits, hemoglobins, reticulocyte counts, and clotting time. Biochemical determinations included serum glutamic oxaloacetic and pyruvic transaminases, urea nitrogen, glucose, lactate, pyruvate, promthrombin time, alkaline phosphatase, lactic acid dehydrogenase, GGT, iron, calcium sodium, potassium, chloride, total protein, creatinine, cholesterol, triglycerides, total lipids, and total and direct bilirubin. Blood samples were submitted to the battery of tests initially and terminally for dogs but only terminally for rats.

Urine analyses were conducted on dogs only. Initial urine specimens were collected by catherterization while terminal specimens were collected by puncture of the urinary bladder during necropsy. Urinary tests included specific gravity, microscopic examination of the sediment, and qualitative analysis for blood, glucose, pH, protein, bilirubin, and

ketones. Tests for urobilinogen were also conducted.

At necropsy, the following tissues were harvested, preserved, and submitted for histopathology: adrenals, aorta, brain, bone marrow, eyes with optic nerves, gall bladder (dogs only), gastrointestinal tract, heart, kidneys, liver, lacrimal glands, mesenteric and axillary (dogs only) lymph nodes, lungs, mammary gland, pancreas, pituitary, prostate, testes with epididymides, ovaries and uterine horns, sciatic nerve, skin with muscle, spleen, trachea, thyroids with parathyroids, urinary bladder, all lesions, and tissue masses. Adrenal, kidney, and liver weights were recorded at necropsy.

No statistical analyses were conducted on dog data or rat clotting times. Prior to analyses, square root transformations were applied to WBC differentials and logarithmic transformations were applied to alkaline phosphatase and SGPT. One-way analyses of variance followed by a t-test with Dunnetts or Scheffe criteria were applied to data demonstrating homogeneous variances as defined by Bartlett's test. An asymptotic F test, followed by Behren's t-test with Cochran's approximation, was used for data demonstrating heterogeneous variances.

GLP: Unknown

Test Substance: Sodium salt of DCA, purity 99.5-100.7%

Results: <u>Rats</u>

Mortality of 2 rats/sex occurred at 2000 mg/kg. Piloerection, tactile-induced vocalization, low body posture, and unthriftiness were exhibited by the males prior to death (study days 60 and 62). The females died on study days 34 and 72, and appeared cachectic and unthrifty prior to death. Other signs of intoxication observed included hindlimb paralysis (26.7% of each sex at 2000 mg/kg) and pollakiuria (13.3% males and 26.7% females at 2000 mg/kg). Hindlimb paralysis first occurred at approximately 2 months on test. One rat of each sex which exhibited hindlimb paralysis during the exposure period appeared to recover completely during the 4-week recovery period.

Reductions in body weights and food consumption occurred in all DCA-treated rats. Both effects were clearly reversed upon cessation of DCA treatment.

Marginal but significant suppressions of erythroid parameters were induced at all dose levels, although bone

marrows and spleens of the treated rats appeared normal histologically. No effects on erythroid parameters were observed during the recovery period.

Blood biochemistries revealed that treated groups of both sexes experienced significant dose-dependent depressions in glucose. Mean concentrations of lactate were reduced (all doses of both sexes) and creatinine was increased in females at 500 mg/kg and males and females at 2000 mg/kg. Treated males also exhibited significantly lower triglycerides (500 and 2000 mg/kg), lower total proteins (all doses), lower calcium (2000 mg/kg), increased total and direct bilirubin (500 and 2000 mg/kg), increased sodium (2000 mg/kg), and increased potassium (2000 mg/kg). All parameters returned to control or baseline levels during the recovery period.

At necropsy, small testes was observed in the 2000 mg/kg males, including those at the 4-week recovery sacrifice. Liver weights of the 500 and 2000 mg/kg females were significantly increased. Mean relative liver weights (all DCA doses of both sexes), kidneys (females at all DCA doses), and adrenals (500 mg/kg males, 2000 mg/kg males and females) were increased along dose dependent lines. Both absolute and relative organ weights approached those of controls at the recovery sacrifice.

Histopathology examinations revealed that brain and testes were the target organs. Brain lesions resembling edema occurred in both sexes with a combined incidence rate of 60% at 125 mg/kg and 100% at both 500 and 2000 mg/kg. These lesions occurred mainly in the cerebrum and to a lesser extent in the cerebellum. In 3/8 rats, the brain lesions persisted after cessation of treatment.

DCA-treated males exhibited testicular germinal epithelial degeneration at 500 mg/kg (40%) and 2000 mg/kg (100%). In all males at 2000 mg/kg, the testes appeared aspermatogenic and contained syncytial giant cells in the germinal epithelium while the epididymis ducts were devoid of spermatozoa. Among the recovery males, 50% exhibited some germinal epithelium regeneration; however, 75% were aspermatogenic and 100% showed loss of germinal epithelium.

Dogs

One female died on day 40 at 75 mg/kg and 1 male died on

day 88 at 100 mg/kg. Prior to death, both animals exhibited anorexia, body weight loss, general weakness, and reduced activity. Other adverse effects attributed to DCA ingestion included limited cases of emesis (75 and 100 mg/kg), bloody stools and paralysis (100 mg/kg), and a high incidence of ocular anomalies (50, 75, and 100 mg/kg). The ocular anomalies included bilateral lentricular opacities, injected bulbar conjunctivae and superficial corneal vascularization, and a tendency for keratoconjunctivitis sicca. The keratoconjunctivitis sicca and the corneal vascularization regressed after DCA treatment was stopped; however, the lentricular opacities were irreversible.

Food consumption was not affected in the males; however, treated females exhibited sharply reduced appetites at all doses. Both sexes exhibited dose-dependent weight losses, which along with the depressed appetites, were reversed upon cessation of DCA treatment.

In both sexes, DCA ingestion was associated with a progressive depression in erythrocyte counts, hematocrits, and hemoglobins at all dose levels. No histopathology was observed in either the bone marrow or spleen. At recovery, these parameters had normalized or markedly improved.

Mean blood levels of pyruvate, lactate, and glucose were substantially and consistently reduced in both sexes of all treated dogs. Calcium and potassium presented marginal results, but tended to be lower among treated animals when compared to baseline values. LDH levels among all treated dogs of both sexes regularly exceeded their own baseline levels and those of the controls. However, the LDH values remained within established normal limits. Following the 5-week recovery period, biochemical values were comparable among all the dose groups and controls.

No effects on urine parameters were observed.

Histopathology findings included the ocular lesions mentioned above, as well as an increased incidence of lung consolidation among treated dogs of both sexes. The authors note that confirmation of nematodes in some lungs of treated dogs may explain the etiology of the lung inflammatory lesions, however, the severity of the pulmonary lesions appeared to have been exacerbated in DCA-treated dogs.

In addition, all treated dogs exhibited slight to moderate vacuolation of white myelinated tracts in the cerebrum and to a much lesser extent in the cerebellum. This lesion was persistent in 2 dogs (1 of each sex) during the recovery period. Prostate glandular atrophy and testicular changes in the germinal epithelium (degeneration, synctical giant cells, vacuolation of Leydig cells) were observed among all treated males and were judged to be dose-dependent. In the recovery males, the prostate appeared normal and there was evidence of germinal epithelium regeneration with spermatogenesis. Increased incidence of hemosiderin-ladened Kupffer cells in the liver (67% at 50 mg/kg; 50% at 75 mg/kg; 83% at 100 mg/kg) and cystic mucousal hyperplasia in the gall bladder (67% at 50 mg/kg; 80% at 75 mg/kg; 100% at 100 mg/kg) were also noted in the histopathology examinations. Both of these abnormalities were still evident at the 5-week recovery examination.

The LOAEL in rats was 125 mg/kg (the lowest dose tested). The LOAEL in dogs was 50 mg/kg (the lowest dose tested). A NOAEL was not established in rats or dogs.

Reference: Katz, R. et al. (1981). Toxicol. Appl. Pharmacol., 57(2):273-287.

Reliability: High because a scientifically defensible or guideline method was used.

**Type:** **90-Day Oral Toxicity Study**

Species/Strain: Dogs/Beagle

Sex/Number: Male and female/5 per sex per group

Exposure Period: 90 days

Frequency of Treatment: Daily

Exposure Levels: 0, 12.5, 39.5, 72 mg/kg

Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Dogs were housed individually in stainless steel cages in animal rooms that were kept at 25±2ºC, 40-60% relative humidity, and a 12-hour light cycle. Food and water were available *ad libitum*.

Due to its low pH and corrosive properties, DCA was neutralized with NaOH to a final pH of 7.4. Dogs received daily oral doses in gelatin capsules. Control dogs received gelatin capsules containing distilled water.

Body weights, clinical signs, and food and water consumption were monitored throughout the study.

Blood samples for hematological and biochemical determinations were collected on Days 0, 15, 30, 45, 60, 75, and 90. The following parameters were assessed or calculated: alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), creatinine, blood urea nitrogen, total bilirubin, calcium, total erythrocyte count, hematocrit, hemoglobin, leucocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and leukocyte differentials.

At the end of the 90-day period, dogs were euthanised. Food was withheld 24 hours prior to sacrifice. At necropsy, all major organs were weighed and gross lesions described. The following tissues were examined microscopically: cerebrum, cerebellum, medulla, salivary gland, pancreas, axillary lymph node, thyroid, parathyroid, trachea, esophagus, heart, colon, jejunum, aorta, stomach, duodenum, ileum, spleen, urinary bladder, lungs, sciatic nerve, spinal cord, kidneys, liver, ovaries/testes, uterus/prostate gland, skin, mammary gland, eyes, sternum/bone marrow, femur, and gall bladder.

Final body weight and organ weights were analyzed with a one-factor analysis of variance (ANOVA) with Tukey's multiple comparison procedure. The assumption of homogeneity of variance was tested by the Levene's test. ANOVA procedures with contrast comparisons were used in the pairwise analyses. A linear trend analysis was also done for each response measure using ANOVA. The pathology lesion data were analyzed by the exact test for trend. A one-tailed Fisher exact test was used in the pairwise comparisons of each dose group with its appropriate control. The distribution of hematology and serum enzyme data contained many extreme data points and therefore no analysis of raw data values was performed. Instead, the numbers of animals outside the normal range were examined. The exact test for trend and the one-tailed Fisher exact test were also used in the analysis.

GLP: Unknown

Test Substance: DCA, purity not reported

Results: One female and 2 males died in the 72 mg/kg group. Deaths

occurred on Days 50, 51, and 74. The postmortem exam revealed that these deaths were likely due to pneumonia and dehydration.

Dyspnea was noted around Day 45 in 4/10 dogs in the 39.5 mg/kg group and 8/10 dogs in the 72 mg/kg group. All dogs in the 72 mg/kg group exhibited severe dyspnea by the end of the study.

Bilateral conjunctivitis accompanied by a slight clear ocular discharge was noted in 24/30 treated dogs in the first month of the study and was occasionally noted in a few control dogs. The conjunctivitis progressed to a pronounced degree of swelling, becoming more purulent in 8/10 high dose dogs. The discharge remained clear throughout the study in the low and mid-dose dogs.

Slight bilateral posterior paresis was observed beginning around Day 50 in 1 female and 2 males in the 72 mg/kg group. Once observed the paralysis persisted, but did not progress until near the end of the study.

Diarrhea was observed sporadically in the 39.5 and 72 mg/kg dose groups. Once noted, the diarrhea became progressively worse. In some dogs, fluid therapy was needed to avoid severe dehydration.

The 72 mg/kg males exhibited a 16% weight loss, the 72 mg/kg females and the 39.5 mg/kg males exhibited a 9% loss, and the 39.5 mg/kg females showed an 11% loss in body weight during the 90-day study. Reduction in food and water consumption was noted in all treated dogs.

Significant decreases in erythrocyte count and hemoglobin levels were observed in the 72 mg/kg males and females. ALT, AST, and LDH values showed significant changes.

At necropsy, several gross changes were observed in the 72 mg/kg groups. The lungs were mottled and showed moderate red discoloration. The kidneys were pale and were discolored yellow-brown. White frothy material was present in the trachea, and the liver showed mild yellow discoloration. Liver weights of both male and female dogs were significantly higher than those of controls at all DCA doses. Percentage kidney weights in 39.5 and 72 mg/kg males and females were significantly increased. The lung

weights of the 72 mg/kg males and females were significantly higher than controls. No significant weight changes were observed in the testes or ovaries. The 72 mg/kg males and females showed increased relative brain weights.

The microscopic exam revealed mild vacuolization of white myelinated tracts in the brain of all DCA-treated dose groups. In some dogs, the vacuolization was present in both cerebrum and cerebellum, while in other dogs it was present in the cerebrum or cerebellum. Vacuolar change was also observed in the medulla and spinal cord of some males and mild meningoencephalitis was present in one 72 mg/kg female. Incidence data for these findings can be found in the table below.

| | 12.5 mg/kg | 39.5 mg/kg | 72 mg/kg |
|---|---|---|---|
| Vacuolization in cerebrum and cerebellum | 0/5 (F)<br>1/5 (M) | 0/5 (F)<br>3/5 (M) | 0/5 (F)<br>1/5 (M) |
| Vacuolization in cerebrum only | 0/5 (F)<br>2/5 (M) | 1/5 (F)<br>0/5 (M) | 1/5 (F)<br>2/5 (M) |
| Vacuolization in cerebellum only | 0/5 (M) | 1/5 (M) | 0/5 (M) |
| Vacuolization in medulla | 0/5 (F) | 0/5 (F) | 1/5 (F) |
| Meningo-encephalitis | 0/5 (F) | 0/5 (F) | 1/5 (F) |
| Vacuolar change – medulla and spinal cord | 0/5 (M) | 5/5 (M) | 3/5 (M) |

Hepatic, lung, pancreatic, and testicular pathologic changes are summarized in the table below.

| | Control | 12.5 mg/kg | 39.5 mg/kg | 72 mg/kg |
|---|---|---|---|---|
| Hepatic Lesions | | | | |
| Vacuolar change | 2/5 (M)<br>2/5 (F) | 4/5 (M)<br>5/5 (F) | 3/5 (M)<br>2/5 (F) | 2/5 (M)<br>2/5 (F) |
| Chronic inflammation | 0/5 (M)<br>0/5 (F) | 0/5 (M)<br>0/5 (F) | 0/5 (M)<br>1/5 (F) | 2/5 (M)<br>2/5 (F) |
| Hemosiderosis | 0/5 (M)<br>0/5 (F) | 1/5 (M)<br>0/5 (F) | 1/5 (M)<br>3/5 (F) | 2/5 (M)<br>3/5 (F) |
| Lung Lesions | | | | |
| Suppurative bronhco-pneumonia | 0/5 (M)<br>0/5 (F) | 0/5 (M)<br>0/5 (F) | 2/5 (M)<br>1/5 (F) | 2/5 (M)<br>4/5 (F) |
| Chronic pneumonia | 1/5 (M)<br>0/5 (F) | 1/5 (M)<br>1/5 (F) | 0/5 (M)<br>3/5 (F) | 1/5 (M)<br>0/5 (F) |
| Granulomatous pneumonia | 0/5 (M)<br>0/5 (F) | 0/5 (M)<br>0/5 (F) | 0/5 (M)<br>0/5 (F) | 0/5 (M)<br>1/5 (F) |
| Edema | 0/5 (M)<br>0/5 (F) | 1/5 (M)<br>0/5 (F) | 2/5 (M)<br>0/5 (F) | 3/5 (M)<br>0/5 (F) |
| Pancreatic Lesions | | | | |
| Chronic inflammation | 0/5 (M)<br>0/5 (F) | 1/5 (M)<br>0/5 (F) | 2/5 (M)<br>1/5 (F) | 2/5 (M)<br>2/5 (F) |
| Acinar degeneration | 0/5 (M)<br>0/5 (F) | 0/5 (M)<br>0/5 (F) | 2/5 (M)<br>1/5 (F) | 2/5 (M)<br>3/5 (F) |
| Testicular Lesions | | | | |
| Degeneration | 0/5 (M) | 4/5 (M) | 5/5 (M) | 5/5 (M) |

Testicular changes featured syntical giant cell formation and degeneration of germinal epithelium. The 39.5 and 72 mg/kg males had an increased severity of these lesions. Prostatic glandular atrophy characterized by a significant reduction of glandular alveoli was noted in the 39.5 and 72 mg/kg groups. Thymic atrophy was observed in most 72 mg/kg males and was characterized by a marked depletion of lymphoid tissue.

A NOAEL was not determined in this study. The LOAEL was 12.5 mg/kg.

Reference: Cicmanec, J. L. et al. (1991). Fundam. Appl. Toxicol., 17(2):376-389.

Reliability: High because a scientifically defensible or guideline method was used.

**Type:** **Chronic Carcinogenicity Study in Rats**

Species/Strain: Rats/Fischer F344

Sex/Number: Male/50-78

Exposure Period: 100-103 weeks

Frequency of Daily

Treatment:

Exposure Levels: Study 1: 0 (NaCl control), 0.05, 0.5, 5 g/L (lowered to 2.5 g/L at 9 weeks, 2.0 g/L at 23 weeks, and 1.0 g/L at 52 weeks)

Study 2: 0 (deionized water), 2.5 g/L (lowered to 1.5 g/L at 8 weeks and 1.0 g/L at 26 weeks)

Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

DCA was dissolved in distilled water to produce the nominal concentration. The pH was adjusted to 6.9-7.1 by the addition of NaOH. Freshly prepared solutions were administered to the animals in water bottles fitted with stoppers and sipper tubes. The drinking water solutions were changed every 5-7 days. The stability of DCA over these time periods was demonstrated by gas chromatography. The drinking water solutions were sampled periodically throughout the study to determine the actual DCA concentrations.

In the first study, the control group received 2 g/L NaCl. DCA doses of 0.05, 0.5, and 5 g/L were initially used. The 5 g/L dose was sequentially lowered to 2.5 g/L at 9 weeks, to 2.0 g/L at 23 weeks, and to 1.0 g/L at 52 weeks due to irreversible, peripheral neuropathy. The rats did not recover and were sacrificed at 60 weeks and were excluded from the study analysis. In the second study, deionized water (vehicle) was administered to the control group. Another group of rats received 2.5 g/L DCA, which was lowered to 1.5 g/L at 8 weeks and to 1.0 g/L at 26 weeks. A mild transient neurotoxicity observed at 2.5 g/L was mostly ameliorated with the lowered DCA concentration and the rats survived to 103 weeks. The NaCl was omitted from the water in the second study because earlier studies had found no significant osmotic effect, altered water consumption, or differences in tumor incidences between water containing NaCl and the deionized water alone.

Rats were 28-30 days of age at the beginning of treatment with mean initial body weights ranging from 59-79 g. Rats were housed 2-3 per polycarbonate cage and provided food and water *ad libitum*. Animal rooms were maintained at 20-22ºC and 40-60% relative humidity on a 12-hour light-dark cycle.

Cageside observations and mortality and morbidity checks were made daily. Body weight, water consumption, and careful physical examinations were periodically measured throughout the study.

In the first study, sacrifices were conducted at 15, 30, 45, 60, and 100 weeks. At the interim sacrifices, body, liver, kidneys, testes, and spleen were weighed and examined for gross lesions. Tissues were harvested, fixed, trimmed, embedded in paraffin, and sectioned. Slides were stained with hematoxylin and eosin. The remaining liver and kidneys were frozen in liquid nitrogen and stored at -70ºC. At the 100-week sacrifice, a complete necropsy was performed. Rats were examined for gross lesions. All gross lesions and representative samples from brain, sciatic nerve, salivary gland, pancreas, pituitary, adrenals, thymus, thyroid, parathyroids, trachea, esophagus, lungs, liver, spleen, skeletal muscle, tongue, heart and aorta, stomach, duodenum, jejunum, ileum, colon, caecum, rectum, kidneys, urinary bladder, prostate, seminal vesicles, testes, preputial gland, mammary gland, femur, nasal cavity, larynx, skin, mesenteric and mandibular lymph nodes were placed in formalin. Liver, kidneys, spleen, and testes were examined microscopically. In addition, a complete pathologic examination was performed on 5 high dose animals from each of the final sacrifices.

In the second study, sacrifices occurred at 14, 26, 52, 78, and 103 weeks. Liver, kidneys, testes, thyroid, stomach, rectum, duodenum, ileum, jejunum, colon, urinary bladder, and spleen were examined for gross lesions. The tissues and lesions were harvested, fixed, processed and examined microscopically as described above.

Tumor prevalence as well as multiplicity was calculated.

Portions of frozen livers were homogenized. The homogenates were centrifuged, the fatty layers removed, and the extract stored at -70ºC until assayed. Cyanide-insensitive Palmitoyl coenzyme A (PCO) was measured according the method of Osumi, T. and T. Hashimoto (1978). J. Biochem., 83:1361-1365.

Five days prior to each scheduled sacrifice, osmotic pumps were implanted subcutaneously in the rats. Autoradiography

using paraffin embedded section from the left liver lobe was performed according to the procedure of Leblond, L. and W. L. Percival (1948). Soc. Exp. Biol. Med. Proc., 67:74 as modified by Gride, W. (1968). Autoradiographic materials and procedures. In: Auto-radiographic Techniques, Ch. 3, pp. 14-33, Prentice-Hall, Inglewood Cliffs, NJ. Slides were coated with emulsion and stored for 5-8 weeks at 4ºC. After developing, the slides were counterstained with hematoxylin. BRDU-labeled nuclei were identified. A minimum of 1000 hepatocytes were examined for incorporation of BRDU over the nuclei. The labeling index (LI) was calculated.

Body weight, organ weight, relative (or corrected) weights, labeling index, PCO, and water consumption were treated as continuous data. These variables were analyzed using a one-way analysis of variance. Detection of some overall effect of treatment groups was followed by pairwise comparisons to controls using appropriate contrasts. If either the homoscedasticity assumption (Levene's test) or the normality assumption (Shapiro-Wilk test) was violated then a non-parametric analysis (an ANOVA on the ranks of the data) was performed, followed by non-parametric (Wilcoxon rank sum) pairwise comparisons with controls. Tests for trend with dose were performed using contrasts in the mean responses.

For liver tumor prevalence, overall differences among treatments and for comparison with controls, respectively, the likelihood ratio $X^2$ test and Fisher's Exact test were used. Similar comparisons involving the counts of tumors per liver were performed using log-rank tests. Trends of tumor prevalence were evaluated using an extension of the Fisher-Irwin test. Trends of tumor counts were evaluated using a log-rank monotone trend test. Survival curves were determined using the product limit estimates of Kaplan-Meier and test for equality of survival curves across strata were performed using the log-rank test.

GLP: Unknown

Test Substance: DCA, purity >99%

Results: Dosing and survival data for the 2 studies are summarized in the tables below. There were no significant differences in animal survival between the control and treatment groups. The unscheduled deaths were primarily due to mononuclear cell leukemia which occured in the neoplasm in the male F344 rat.

| | Control | 0.05 g/L | 0.5 g/L |
|---|---|---|---|
| Measured concentration | - | - | 0.42 |
| Water consumption (mL/kg/day) | 76.9 | 85.1 | 95.8 |
| Mean daily dose (mg/kg/day) | - | 3.6 | 40.2 |
| Number of animals at study start | 50 | 60 | 60 |
| Number of unscheduled deaths | 6 | 12 | 10 |
| Number of animals at interim sacrifices | 21 | 27 | 27 |
| Number of animals at final sacrifice | 23 | 21 | 23 |
| Drinking water solutions prepared by diluting 0.5 g/L were not analyzed | | | |

| | Control | 2.5 g/L |
|---|---|---|
| Measured concentration | - | 1.61 |
| Water consumption (mL/kg/day) | 61.7 | 86.4 |
| Mean daily dose (mg/kg/day) | - | 139.1 |
| Number of animals at study start | 78 | 78 |
| Number of unscheduled deaths | 17 | 23 |
| Number of animals at interim sacrifices | 28 | 27 |
| Number of animals at final sacrifice | 33 | 28 |

In the first study, there were no significant differences in the final body weights of the rats. In the second study after 103 weeks, the mean body weight of the animals exposed to 1.6 mg/L DCA was significantly reduced to 73% of the control value (308 vs. 424 g).

In the first study, no differences in the absolute and relative weights of liver, kidney, and spleen were noted for the 0.05 or 0.5 g/L groups. Absolute and relative testicular weights were mildly increased in the 0.5 g/L group. In the second study, the absolute weights of liver, kidneys, and spleen of the 1.6 g/L rats did not differ from controls. The relative

liver and kidney weights of the 1.6 g/L group were increased due to their depressed body weights. The absolute testes weight at 78 weeks for the 1.6 g/L group was depressed. The relative testes weight was lower, but not significantly.

Based upon the pathologic examination, DCA induced observable signs of toxicity in the nervous system, liver, and myocardium. However, treatment-related neoplastic lesions were observed only in the liver. The various phenotypically altered hepatic foci (AHF; basophilic, eosinophilic, clear cell or mixed cell) were regularly observed. The AHF contained cells that were small and arranged in irregularity formed hepatic plates and were few in number. The prevalence of AHF did not appear to differ between control and treated animals. Non-neoplastic changes diagnosed from gross observations and pathologic examination were not considered treatment related and were consistent with aging changes previously observed in F344 rats. Hepatocellular cytoplasmic vacuolization was more prominent in the DCA dosed animals. Several degenerative hepatic changes such as sinusoidal dilation and cystic degerneration were accentuated somewhat in the treated animals. All of the non-hepatic neoplastic lesions observed were considered spontaneous for F344 male rats. None of the neoplastic lesions in other organs exceeded the percent incidence when compared to a historical control database.

Testicular interstitial cell tumors were seen in 100% of the animals in the 0.05 and 0.5 g/L groups versus 97% for the NaCl control group and 100% of the rats in the water control and 1.6 g/L groups. The incidence of mononuclear cell leukemia was 24% for the NaCl control group, 10% in the 0.05 g/L group, and 43% in the 0.5 g/L group. The incidence of mononuclear cell leukemia in the water control and 1.6 g/L groups was 9 and 11%, respectively. Kidney neoplasms were not apparent in the NaCl control group or in the 0.05 and 0.5 g/L groups. One DCA treated animal had a renal tubular cell adenoma.

The following table summarizes the prevalence of the hepatocellular lesions observed.

| | Control | 0.05 g/L | 0.5 g/L | Control | 1.6 g/L |
|---|---|---|---|---|---|
| No. examineda | 23 | 26 | 29 | 33 | 28 |
| Hyperplastic nodule | 4.4b | 0 | 10.3 | 3.0 | 3.6 |
| Adenoma | 4.4 | 0 | 17.2 | 0 | 10.7 |
| Carcinoma | 0 | 0 | 10.3 | 3.0 | 21.4 |
| Neoplasia* | 4.4 | 0 | 24.1 | 3.0 | 28.6 |
| Proliferative lesions** | 8.7 | 0 | 34.9 | 6.1 | 32.1 |

a = Animals that survived 78 weeks.
b = Numbers represent the percent of animals with at least 1 lesion.
* = Combined adenoma and carcinoma
** = Combined hyperplastic nodules, adenoma, and carcinoma

Rats exposed to 0.5 g/L DCA had an increased multiplicity for neoplasia and total proliferative lesions. Rats treated with 1.6 g/L DCA also exhibited an increased multiplicity. The following table shows the effect of the multiplicity of the hepatocellular proliferative lesions.

| | Control | 0.05 g/L | 0.5 g/L | Control | 1.6 g/L |
|---|---|---|---|---|---|
| No. examineda | 23 | 26 | 29 | 33 | 28 |
| Hyperplastic nodule | 0.04 | | 0.10 | 0.03 | 0.04 |
| Adenoma | 0.04 | | 0.21 | | 0.11 |
| Carcinoma | | | 0.10 | 0.03 | 0.25 |
| Neoplasia* | 0.04 | | 0.31 | 0.03 | 0.36 |
| Proliferative lesions** | 0.09 | | 0.41 | 0.06 | 0.39 |

Multiplicity = number of lesions/animal
a = Animals that survived 78 weeks.
* = Combined adenoma and carcinoma
** = Combined hyperplastic nodules, adenoma, and carcinoma

DCA administered in the drinking water did not increase PCO activity above the NaCl control value at 15, 30, and 60 weeks. DCA enhanced the PCO activity in the 1.6 g/L group relative to the water control at 14, 26, and 78 weeks.

Neither 0.05 or 0.5 g/L DCA altered the hepatocyte labeling index (LI) measured in hepatocytes outside of the various hepatoproliferative lesions when compared to the NaCl control group at any time period. At 14 weeks, 1.6 g/L DCA depressed the LI to 41% of the control value (1.04 versus 2.53). The LI measured at the other time points, while depressed, did not differ significant from the control value:

26 weeks (28% of control); 52 weeks (9% of control); and 78 weeks (57% of control).

The authors stated that this data demonstrates that DCA is a hepatocellular carcinogen in the male F344 rat. The authors stated that a NOEL of 0.05 g/L for DCA carcinogenicity was established in this study.

Reference: DeAngelo, A. B. et al. (1996). Toxicology, 114:207-221.

Reliability: High because a scientifically defensible or guideline method was used.

**Additional References for Repeated Dose Toxicity:**

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DeAngelo, A. B. et al. (1989). Toxicol. Appl. Pharmacol., 101:285-298.

Kato-Weinstein, J. et al. (1998). Toxicology, 130:141-154.

Sanchez, I. M. and R. J. Bull (1990). Toxicology, 64(1):33-46 (cited in EPA (2003). EPA 635/R-03/007, "Toxicological Review of Dichloroacetic Acid in support of IRIS" (August)).

Evans, O. B. and P. W. Stacpoole (1982). Biochem. Pharmacol., 31:1295-1300 (cited in EPA (2003). EPA 635/R-03/007, "Toxicological Review of Dichloroacetic Acid in support of IRIS" (August)).

Davis, M. E. (1986). Environ. Health Perspect., 69:209-214 (cited in cited in EPA (2003). EPA 635/R-03/007, "Toxicological Review of Dichloroacetic Acid in support of IRIS" (August)).

Ribes, G. et al. (1979). Diabetes, 28:852-857 (cited in EPA (2003). EPA 635/R-03/007, "Toxicological Review of Dichloroacetic Acid in support of IRIS" (August)).

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Yount, E. A. et al. (1982). J. Pharmacol. Exp. Ther., 222(2):501-508.

Bhat, H. K. et al. (1991). Fundam. Appl. Toxicol., 17:240-253.

Mather, G. G. et al. (1990). Toxicology, 64(1):71-80.

Moser, V. C. et al. (1999). Neurotox. Teratol., 21(6):719-731.

DeAngelo, A. B. et al. (1991). Fundam. Appl. Toxicol., 16(2):337-347.

DeAngelo, A. B. and L. P. McMillian (1990). Water Chlorination, 6:193-199 (CCRIS/4016).

DeAngelo, A. B. et al. (1999). J. Toxicol. Environ. Health, 58:485-507.

Richmond, R. E. et al. (1995). Cancer Lett., 92:67-76.

Herren-Freund et al. (1987). Tox. Appl. Pharm., 90(2):183-189.

Herren-Freund, M. A. et al. (1986). Proceed. Am. Assoc. Cancer Res., 27:357.

Bull, R. J. et al. (1990). Toxicology, 63:341-359.

Bull, R. J. (1989). U.S. Air Force Office of Scientific Research, NTIS AD-A214501 (CCRIS/4016).

US EPA (1991). Toxicology of the chloroacetic acids by-products of the drinking water disinfection process. II. The comparative carcinogenicity of dichloroacetic acid and trichloroacetic acid: Implication in risk assessment, Document No. HERL-0820, US EPA Health Effects Research Laboratory, Research Triangle Park, NC (cited in EPA (2003). EPA 635/R-03/007, "Toxicological Review of Dichloroacetic Acid in support of IRIS" (August)).

Daniel, F. B. et al. (1992). Fundam. Appl. Tox., 19(2):159-168.

Pereira, M. A. and J. B. Phelps (1996). Cancer Lett., 102:133-141.

Pereira, M. A. (1996). Fundam. Appl. Toxicol., 31(2):192-199.

Yount, E. A. et al. (1981). Fed. Proceed., 40(6):485.

Stacpoole, P. W. et al. (1979). N. Engl. J. Med., 300(7):372.

## 5.3 Developmental Toxicity

| | |
|---|---|
| Species/Strain: | Rats/Long-Evans |
| Sex/Number: | Female/20 per group |
| Route of Administration: | Oral gavage |
| Exposure Period: | Gestation Days 6-15 |
| Exposure Levels: | 0, 900, 1400, 1900, 2400 mg/kg/day<br>0, 14, 140, 400 mg/kg/day |
| Method: | No specific test guideline was reported; however, a |

scientifically defensible approach was used to conduct the study.

Female rats, approximately 65-80 days old upon arrival, were housed in groups of 3 in plastic cages with corn cob bedding and maintained on food and water *ad libitum*. Animal rooms were maintained at 21-23°C and 40-60% humidity with a 12-hour light cycle. Females in proestrus were selected from the breeding colony by vaginal cytology, and placed with a male (1 female:1 male) overnight. The female was checked for the presence of sperm the following morning. Sperm-positive females were considered to be in day 0 of pregnancy and were housed singly for the duration of the study.

DCA was dissolved in water and adjusted to pH 7 with sodium hydroxide. Dosing solutions were prepared daily, at a dosing volume of 10 mL/kg. The purity and stability of the dosing solutions was confirmed using ion chromatography.

Two separate studies were conducted. The first study was conducted with dose levels of 0, 900, 1400, 1900, and 2400 mg/kg/day. The second study was conducted with dose levels of 0, 14, 140, and 400 mg/kg/day (calculated as the free acid). Distilled water served as the vehicle control.

Clinical signs and body weights were recorded periodically throughout the study. Females dying prematurely were subjected to a gross necropsy. On gestation day 20, dams were killed and their livers, spleens, and kidneys were removed and weighed. Corpora lutea were counted. The uterine horns were examined for the number and location of fetuses or resorption sites. The fetuses were removed, weighed, measured for crown-rump length, sexed, and evaluated for external abnormalities. Two-thirds of each litter were fixed in Bouin's solution for free-hand razor blade sectioning. The remaining fetuses were fixed, double stained, and examined for skeletal abnormalities.

Maternal body and organ weights (first study), mean fetal weights and crown-rump lengths, and litter sizes (first study) were analyzed using the ANOVA procedure. Differences between the dose groups and control were examined using pairwise contrasts (Winer, B. J. (1971). Statistical Principles in Experimental Design, McGraw-Hill Book Co., New York). The presence of a significant dose response was

tested with linear regression. The proportions of maternal deaths and pregnancies were compared using the Z-test for differences between proportions (Walpole, R. E. and R. H. Myers (1978). Probability and Statistics for Engineers and Scientists, 2nd ed., Macmillan Publishing Co., New York). Organ weights (second study), litter sizes (second study), sex ratios, and percentages of resorptions and fetuses with abnormalities were evaluated with the Kruskal-Wallis test for overall differences, and with pairwise Mann-Whitney tests to compare each treatment to control. Jonckheere's test was used to analyze for a dose-related response.

GLP: Unknown

Test Substance: DCA, purity > 99%

Results: Maternal deaths occurred at = 1400 mg/kg. The animals died about midway through the dosing period. Reduced maternal body weight gain was observed at = 140 mg/kg. Maternal liver, spleen, and kidney weights were also elevated indicating an adaptive response at higher doses. Pregnancy rates were high in all dose groups except 900 mg/kg. This result appeared to be unrelated to treatment. The total number of implants per litter were unaltered by treatment with the exception of a spurious reduction at 400 mg/kg. Preimplantation loss was unaffected by treatment.

In the first study, the mean number of live fetuses per litter was significantly reduced, and overall post-implantation loss was statistically elevated over the control values at all doses. These parameters were unaffected in the second study. Fetal body weight and crown-rump length were lower than controls at = 400 mg/kg. The number of male to female surviving fetuses was increased at 2400 mg/kg. Since no dose-related trend was observed in sex ratio at the lower doses, the authors stated that it was unclear if the change at 2400 mg/kg was treatment-related. A summary of maternal and reproductive outcomes is provided in the tables below:

| Treatment (mg/kg) | Pregnant/ Sperm positive | Deaths | Viable Litters | Body Weight (g) on GD 20 | % Wt gain |
|---|---|---|---|---|---|
| 0 | 20/20 | 0 | 20 | 356.8 | 48.6 |
| 900 | 16/19 | 0 | 16 | 318.9 | 39.2 |
| 1400 | 19/19 | 1 | 18 | 334.6 | 36.2 |
| 1900 | 19/19 | 2 | 17 | 334.5 | 36.9 |
| 2400 | 21/21 | 5a | 16 | 318.1 | 31.5 |
| | | | | | |
| 0 | 19/19 | 0 | 19 | 343.0 | 51.5 |
| 14 | 18/19 | 0 | 18 | 348.5 | 49.7 |
| 140 | 19/20 | 0 | 19 | 337.2 | 48.0 |
| 400 | 19/19 | 0 | 19 | 326.8 | 43.6 |
| a = one death was accidental. | | | | | |

| Treatment (mg/kg) | Total implants per litter | Mean live fetuses per litter | % Post-implantation loss |
|---|---|---|---|
| 0 | 13.9 | 13.2 | 5.5 |
| 900 | 14.1 | 12.3 | 12.7 |
| 1400 | 14.2 | 12.4 | 12.6 |
| 1900 | 14.3 | 12.2 | 13.9 |
| 2400 | 14.8 | 9.6 | 33.8 |
| | | | |
| 0 | 14.3 | 12.8 | 10 |
| 14 | 12.8 | 12.2 | 4.3 |
| 140 | 14.3 | 13.3 | 7.9 |
| 400 | 13.4 | 12.5 | 6.3 |

| Treatment (mg/kg) | Fetal crown-rump length (cm) | | Fetal body weight (g) | | M/F sex ratio |
|---|---|---|---|---|---|
| | M | F | M | F | |
| 0 | 3.48 | 3.39 | 3.65 | 3.44 | 1.58 |
| 900 | 3.13 | 3.10 | 3.14 | 2.96 | 1.13 |
| 1400 | 3.10 | 3.06 | 2.96 | 2.83 | 1.33 |
| 1900 | 3.07 | 2.99 | 2.83 | 2.70 | 1.12 |
| 2400 | 2.99 | 2.95 | 2.72 | 2.59 | 2.32 |
| | | | | | |
| 0 | 3.62 | 3.55 | 3.68 | 3.49 | 0.82 |
| 14 | 3.64 | 3.59 | 3.75 | 3.60 | 1.26 |
| 140 | 3.56 | 3.49 | 3.60 | 3.46 | 1.17 |
| 400 | 3.46 | 3.38 | 3.43 | 3.27 | 1.18 |

No malformations were observed in 507 control fetuses from 39 litters. Dose-related increases were seen in external, total soft tissue, cardiovascular, urogenital, and orbital malformations. The most frequent abnormality seen was in the cardiovascular system – primarily a defect between the

right ventricle and the ascending aorta, found in 158 fetuses. Other cardiovascular anomalies included levocardia and interventricular septal defect. A summary of fetal malformations is provided in the table below.

| | Treatment (mg/kg) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 14 | 140 | 400 | 900 | 1400 | 1900 | 2400 |
| Cardiovascular | | | | | | | |
| A | 1 | 2(2) | 12(6) | 10(6) | 27(11) | 47(14) | 59(16) |
| B | 0 | 0 | 0 | 2(2) | 4(3) | 7(5) | 8(6) |
| C | 0 | 0 | 0 | 0 | 4(3) | 14(8) | 13(9) |
| Urogential | | | | | | | |
| D | 0 | 1 | 0 | 2(2) | 6(5) | 6(3) | 10(7) |

Values represent number of fetuses (number of litters) affected.
A = Defect between ascending aorta and right ventricle
B = Levocardia
C = Interventricular septal defect
D = Hydronephrosis

The NOEL for maternal and fetal effects was 14 mg/kg/day. Maternal toxicity was observed at = 140 mg/kg. Lower fetal weight and length and increased soft tissue malformations (cardiovascular system and ascending aorta and right ventricle) were observed at = 140 mg/kg.

The developing fetus was not uniquely sensitive to DCA administration.

Reference: Smith, M. K. et al. (1992). Teratology, 46(3):217-223.
Smith, M. K. et al. (1989). Teratology, 39(5):482.
Randall, J. L. et al. (1991). Teratology, 43(5):454.

Reliability: High because a scientifically defensible or guideline method was used.

**Additional References for Developmental Toxicity:**

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Epstein, D. L. et al. (1992). Teratology 46(3):225-235.

Epstein, D. L. et al. (1990). Teratology, 41(5):553.

Narotsky, M. G. et al. (1996). Teratology, 53(2):96-97.

## 5.4 Reproductive Toxicity

Species/Strain: Rats/Sprague Dawley

| | |
|---|---|
| Sex/Number: | Male/8 per group for the acute study; 6-8 per group in the repeated dose study |
| Route of Administration: | Oral gavage |
| Exposure Period: | Single dose – acute study<br>2, 5, 9, or 14 days – repeated dose study |
| Frequency of Treatment: | See above |
| Exposure Levels: | 0, 1500, 3000 mg/kg for the acute study<br>0, 18, 54, 160, 480, 1440 mg/kg for the repeated dose study |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. |

Rats were housed 2 per cage. Animal rooms were kept at 22±1ºC and 50±10% relative humidity. A 12-hour light/dark cycle was used.

The test substance was dissolved in distilled water and the solutions were adjusted to ~ pH 6.5 with NaOH. Rats were given a single dose of 0 (water control), 1500 or 3000 mg/kg DCA or daily doses of 0, 18, 54, 160, 480, 1440 mg/kg DCA.

Necropsies were performed 2, 14, and 28 days after the single dose of DCA. In the multiple dose study, rats were necropsied 24 hours after the last of 2 daily doses of 0 and 1440 mg/kg, 5 doses of 0, 480, and 1440 mg/kg, 9 doses of 0, 160, 480, and 1440 mg/kg, and 14 doses of 0, 18, 54, 160, 480, and 1440 mg/kg.

Cardiac blood was collected for the testosterone assay. In the single-dose studies, the right testes and epididymides were immersion-fixed in Bouin's solution. In the repeated dose studies, the right testes and epididymes were fixed *in situ* by vascular perfusion in 4 DCA-treated animals from each treatment and 2 controls at each necropsy time point. The right-side organs of nonperfused animals were immersion-fixed in Bouin's solution. Tissues were embedded, sectioned, and stained for examination by light microscopy. In both acute and repeated dose studies, the left testes and epididymides were excised for sperm studies.

Serum testosterone was measured. The left reproductive tracts were used to determine testis and epidimis weights, testicular sperm head counts, and epididymal sperm counts.

Sperm from the caput epididymidis and cauda epididymidis were classified for morphologic anomalies (500 from each region).

For sperm motion analysis, sperm collected from the cauda epididymidis were dispersed in modified Hank's balanced salt solution. Sperm motion parameters were analyzed from videotaped images with an Integrated Visual Optical System (IVOS). Sperm samples were loaded into microslides and 6-13 fields along each microslide were videotaped. Fields were analyzed for each rat until 100 motile cells were tracked or all the fields were analyzed. The motile and nonmotile counts for percent motile were done manually from the playback screen on the IVOS. The percentage of progressively motile sperm was calculated.

Statistical analyses were performed with the Statistical Analysis System. Endpoints were analyzed using the general linear models procedure. Organ weights were subjected to analysis of covariance using the final body weight as the covariant. Sperm measures were analyzed by one-way ANOVA. Pre-planned two-tailed t-test comparisons between control and treated groups were made using the least square means.

GLP: Unknown

Test Substance: DCA, purity = 99%

Results: Acute Study:

No signs of DCA-induced toxicity were observed after single doses of 1500 or 3000 mg/kg. Body weights were not statistically different from controls; however, body weight gain relative to controls was decreased on days 2-9 in the 1500 mg/kg rats and days 9 and 14 in 3000 mg/kg rats.

Delayed spermiation and altered resorption of residual bodies were observed in rats given single doses of 1500 and 3000 mg/kg. Effects persisted to varying degrees on post-treatment days 2, 14, and 28.

Repeated-Dose Studies:

No signs of toxicity were observed in animals exposed to doses of 18 – 1440 mg/kg for 14 days. Body weights were transiently decreased in rats dosed with 1440 mg/kg that were killed on day 2. The body weights were not statistically different from controls in rats killed on days 5 and 9, but were decreased in rats dosed with 480 and 1440 mg/kg that were killed on day 14.

| | |
|---|---|
| | Delayed spermiation and formation of atypical residual bodies were observed on days 2, 5, 9, and 14 in rats dosed daily with 1440, 480, 160, or 54 mg/kg, respectively. Distorted sperm heads and acrosomes were observed in step 15 spermatids after doses of 480 and 1440 mg/kg after 14 days. Decreases in the percentage of motile sperm were observed after 9 days at doses of 480 and 1440 mg/kg, and after 14 days at 160 mg/kg. Increased numbers of fused epididymal sperm were observed on days 5, 9, and 14 in rats dosed with 1440, 480, and 160 mg/kg, respectively. Other morphologic abnormalities occurred at 160 mg/kg and higher. On day 14, a significant decrease in epididymal weight was observed at 480 and 1440 mg/kg, and epididymal sperm count was decreased at 160 mg/kg and higher. |
| Reference: | Linder, R. E. et al. (1997). Reproduct. Toxicol., 11(5):681-686. |
| Reliability: | Medium because a suboptimal study design was used. |

**Additional References for Reproductive Toxicity:**

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Bhat, H. K. et al. (1991). Fundam. Appl. Toxicol., 17:240-253.

Katz, R. et al. (1981). Toxicol. Appl. Pharmacol., 57:273-287.

Toth, G. P. et al. (1992). Fundam. Appl. Toxicol., 19:57-63.

Cicmanec, J. L. et al. (1991). Fundam. Appl. Toxicol., 17:376-389.

Yount, E. A. et al. (1982). J. Pharmacol. Exp. Ther., 222(2):501-508.

Stacpoole, P. W. et al. (1990). Fundam. Appl. Toxicol., 14(2):327-337.

## 5.5 Genetic Toxicity

| | |
|---|---|
| **Type:** | **Prophage-induction assay**<br>***In vitro* Bacterial Reverse Mutation Assay**<br>**Molecular analysis of *Salmonella* revertants** |
| Tester Strain: | Prophage-induction assay: *E. coli* B/r<br>*Salmonella* mutagenicity assay: T100<br>Molecular analysis of *Salmonella* revertants: TA100 |

| | |
|---|---|
| Exogenous Metabolic Activation: | With and without Aroclor®-induced rat liver S9 |
| Exposure Concentrations: | Prophage-induction assay (-S9): 0-5 mg/mL<br>Prophage-induction assay (+S9): 0-10 mg/mL<br>*Salmonella* mutagenicity assay (-S9): 0-600 ppm<br>*Salmonella* mutagenicity assay (+S9): 0-600 ppm |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>Prophage-induction assay:<br>The lambda lysogen $WP2_s$(lambda) was derived from *E. coli* B/r. The indicator strain was TH-008 (Streptomycin$^r$). The assay was performed as described in DeMarini and Brooks, 1992 (DeMarini, D. M. and H. G. Brooks (1992). Environ. Mol. Mutagen., 19:98-111). Test substances were evaluated up to a maximum concentration of 10% (v/v).<br>2-Nitrofluorene (2-NF) was used as the positive control for the trials without metabolic activation and 2-aminoanthracene (2-AA) was used as the positive control for the trials with metabolic activation.<br><br>The first well in a dilution series of a 96-well microtiter plate received supplemented minimal medium and either test compound or medium control. The remaining wells received medium, and 2-fold serial dilutions of the test compound or controls were made down the columns of each plate. Each well was inoculated with a resuspended log-phase culture of $WP2_s$(lambda) and medium or S9 mix. After incubation overnight at 37°C, the wells were scored for turbidity. Turbid wells indicated cell growth and clear wells indicated cytotoxicity and/or inhibition of cell growth. The concentration of lambda bacteriophage was determined by sampling at least the first 5 turbid wells adjacent to a clear well. A sample from a well was diluted, plated onto indicator cells, and incubated overnight at 37°C. Plaque-forming units (PFU or plaques) were counted by hand. The dilution tubes were sampled and diluted in duplicate and all experiments were performed at least twice.<br><br>*Salmonella* mutagenicity assay (bag vaporization method):<br>The vaporization technique was performed as described by Hughes et al., 1987 (Hughes, T. J. et al. (1987). Environ. Mutagen., 9:421-441) with the following modifications. Top agar containing an overnight culture of strain TA100 (± S9 |

mix) was poured onto minimal medium in a glass petri dish. After the top agar had hardened, the bottom and top parts of the petri dish were placed against each other, and the assembly was inserted into a Tedlar® bag of known volume (600-800 mL) with the inverted top of the dish directly under the septum of the bag. The bag was then sealed, and various amounts of test substance were injected through a septum on the bag into the inverted top of the petri dish. The bag was placed in a 37°C incubator for 24 hours. The bag was then opened, the 2 halves of the petri dish were reassembled, and the inverted plate was placed back into the incubator for an additional 48 hours. Colonies were counted by an automatic colony counter. Each petri plate was in a separate bag, and 2 plates were exposed at each concentration of chemical tested. All experiments were performed at least twice. Sodium azide (without metabolic activation) and 2-AA (with metabolic activation) were used as positive controls. A reproducible, 2-fold increase in revertants/plate relative to the background was considered a positive response.

Molecular analysis of *Salmonella* revertants:
Approximately 1200 revertants of TA100 (at least 200 from each treatment group) were analyzed using the colony probe hybridization procedure developed by Cebula and Koch, 1990 (Cebula, T. A. and W. H. Koch (1990). Mutat. Res., 229:79-87) with the modification of Shelton et al., 1994 (Shelton, M. L. et al. (1994). Mutat. Res., 323:35-39). These were compared to approximately 600 background revertants from laboratory historical controls. The selected revertants were streaked onto minimal medium supplemented with biotin and incubated for 2 days at 37°C in order to purify each revertant clone from any nonrevertant cells that might have been transferred from the background lawn. Two independent hybridizations were performed with each probe on each revertant in order to confirm the revertant genotype. Six revertant strains of known genotype were used as positive controls. Mutation spectra were compared by Chi-square analysis using the Stat-Sak program.

GLP: Unknown

Test Substance: DCA, purity 99%

Results: Positive

Remarks: In the Microscreen prophage-induction assay, DCA with metabolic activation was clearly genotoxic producing 3.7-4.3-fold increased in PFU/plate relative to the background values in the 2 experiments. The lowest effective concentration that produced a 3-fold increase in

PFU/plate relative to the background was ~ 2 mg/mL. DCA produced less than a 3-fold increase in PFU/plate in the absence of S9.

DCA was positive in TA100 with activation at 200 ppm and positive without metabolic activation at 600 ppm.

Chi-square analysis indicated that DCA mutation spectra were significantly different from the background mutation spectra. DCA induced primarily GC to AT transitions.

Reference: DeMarini, D. M. et al. (1994). Mutagenesis, 9(5):429-437.

Reliability: High because a scientifically defensible or guideline method was used.

**Type:** ***In vitro* Bacterial Reverse Mutation Assay**

Tester Strain: *Salmonella typhimurium* strains TA102 and TA2638
*E. coli* strains WP2/pKM101 and WP2 *uvr*A/pKM101

Exogenous Metabolic Activation: With and without rat liver S9 (phenobarbital and 5,6-benzoflavone-induced)

Exposure Concentrations: 0, 313, 625, 1250, 2500, 5000 µg/plate

Method: The procedures used in the test were based on the plate incorporation method with or without metabolic activation as described by Maron, D. M. and B. M. Ames (1983). Mutat. Res., 113:173-215.

Each bacterial strain was inoculated from the original stock cultures into nutrient broth, supplemented with 2 µg/mL tetracycline for TA102, and cultured. Within 2 hours of the end of the growth culture period, cultures were prepared. Culture, test material, phosphate buffer or S9 mix, and amino acid-supplemented molten soft agar were mixed and overlaid on a minimal glucose agar plate. The S9 mix contained 10% of S9 fraction. Plates were incubated at 37ºC for 48 hours and colonies were counted by automatic colony counters, manually, or both depending on which laboratory conducted the test. DCA was tested in at least 2 independent experiments using 5 dose levels and 3 plates per dose. The tests were performed in 2 independent laboratories.

Positive control substances used in the experiment included mitomycin C (MMC), 1-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, and 2-aminoanthracene.

The results were analyzed for statistical significance using a

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| | linear regression test. Doses with observed cytotoxicity, which was judged by toxicity to the background lawn and/or a reduction in the number of revertant colonies, were excluded from the statistical analysis. |
| GLP: | Unknown |
| Test Substance: | DCA, purity not reported |
| Results: | Negative |
| Remarks: | DCA was negative in *Salmonella* TA102 and TA2638 with activation and negative in *E. coli* WP2 (PKM101) and WP2 *uvr*A (PKM101) with activation. |
| Reference: | Watanabe, K. et al. (1996). Mutat. Res., 361(2-3):143-155. |
| Reliability: | High because a scientifically defensible or guideline method was used. |

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| **Type:** | ***In vitro* Bacterial Reverse Mutation Assay** |
| Tester Strain: | *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537<br>*E. coli* WP2 *uvr*A |
| Exogenous Metabolic Activation: | With and without rat liver S9 |
| Exposure Concentrations: | 0, 333, 667, 1000, 3330, 5000 µg/plate |
| Method: | No specific test guideline was reported. |
| | Experiments were conducted on 2-layer plates. The lower layer was Vogel-Bonner minimal medium E plus agar and glucose. The upper layer was agar, sodium chloride, supplemented with histidine/biotin or tryptophan. The S9 mixture contained water, sodium phosphate buffer, glucose 6-phosphate, nicotinamide adenine dinucleotide phosphate (NADP), potassium chloride, magnesium chloride, and 10% S9. |
| | Positive control substances used in the experiment included 2-aminoanthracene, 2-nitrofluorene, sodium azide, ICR-191, and 4-nitroquinoline-N-oxide. |
| | All tests were conducted in triplicate. Aliquots of the culture were used to quantify the number of spontaneous revertants for both bacterial species. Background lawn was evaluated for evidence of cytotoxicity. Revertant colonies were counted manually, or using an automated colony counter. |
| GLP: | Yes |
| Test Substance: | DCA, purity > 99.5% |
| Results: | Negative |

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| Remarks: | No cytotoxicity was observed in dose rangefinding studies up to 5 mg per plate using *Salmonella* strain TA100 and *E. coli* WP2*uvr*A. |
| Reference: | Fox, A. W. et al. (1996). Fundam. Appl. Toxicol., 32:87-95. |
| Reliability: | Medium because a suboptimal study design was used. |

**Additional References for *In vitro* Bacterial Reverse Mutation Assay:**

Data from these additional sources support the negative study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Matsuda, H. et al. (1991). Sci. Total Environ., 103:141-149.

Kohan, M. J. et al. (1998). Environ. Mol. Mutagen., 31(Suppl. 29):36.

Waskell, L. (1978). Mutat. Res., 57:141-143.

Saito, H. et al. (1995). Kankyo Henigen Kenkyu, 17(2):169-177.

Data from these additional sources support the positive study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Giller, S. et al. (1997). Mutagenesis, 12(5):321-328.

Herbert, V. et al. (1980). Am. J. Clin. Nutr., 33:1179-1182.

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| **Type:** | ***In vitro* DNA Repair Test (*umu* test)** |
| Cell Type: | *Salmonella typhimurium* strain TA1535/pSK1002 |
| Exogenous Metabolic Activation: | With and without rat liver S9 (phenobarbital and 5,6-benzoflavone-induced) |
| Exposure Concentrations: | 58.5 µg/mL, other dose levels not reported |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>*Salmonella* was incubated in Luria broth (LB) at 37ºC for 16 hours with shaking. The culture was diluted 50-fold into TGA medium and incubated at 37ºC for 3 hours. The culture was subdivided into 4.8 mL portions in test tubes and DCA was added to each tube. Either S9 or phosphate buffer was added to the tubes. The test tubes were incubated for 2 hours at 37ºC with shaking, followed by the measurement of |

the level of ß-galactosidase activity. ß-Galactosidase activity was determined by a modified method of Miller, J. H. (1972). Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, p. 352. Fractions of the culture were diluted with Z-buffer. The bacterial cells were made permeable to the chromagenic substrate for ß-galactosidase by adding SDS and chloroform and mixing vigorously. The enzyme reaction was initiated by addition of 2-nitrophenyl-ß-D-galactopyranoside solution at 28ºC. After 15 minutes, the reaction was stopped by adding $Na_2CO_3$. The absorbance at $OD_{420}$ and $OD_{550}$ was measured by a spectrometer.

Net genotoxicity of the sample was calculated by subtracting the base value, B (value of the blank in each time), from the genotoxicity of the sample, A, at that time. If the value of (A-B)/B was over 2 then the substance was considered strongly positive. If that value was between 1 and 2, then the substance was considered positive. If the value was between 0.5 and 1, then it was considered weakly positive, and if the value was < 0.5, the substance was considered negative.

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| GLP: | Unknown |
| Test Substance: | DCA, purity not reported |
| Results: | Negative without S9. Positive with S9 at 58.5 µg/mL. |
| Remarks: | No additional data. |
| Reference: | Ono, Y. et al. (1991). Wat. Sci. Tech., 23:329-338. |
| Reliability: | High because a scientifically defensible or guideline method was used. |

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| **Type:** | ***In vitro* Clastogenicity Studies** (Mouse Lymphoma Forward Mutation Assay, Clastogenicity Assay, and Micronucleus Test) |
| Cell Type: | TK+/- -3.7.2C heterozygote of the L5178Y mouse lymphoma cell line |
| Exogenous Metabolic Activation: | Without metabolic activation |
| Exposure Concentrations: | Experiment 1: 0, 100, 150, 600, 800, 1000 µg/mL<br>Experiment 2: 0, 200, 300, 400, 500, 600, 700, 800, 900 µg/mL |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. |

Cells were centrifuged and suspended at a concentration of

0.6 x$10^6$ cells/mL in culture medium supplemented with horse serum. 6x$10^6$ cells were placed in polystyrene tubes, DCA was added, tubes were gassed with 5% $CO_2$ in air and sealed for the duration of treatment. Multiple independent experiments were performed. The cell culture tubes were placed on a roller drum and incubated at 37ºC. At the end of the 4-hour treatment period, the cell cultures were centrifuged, washed twice with fresh medium, resuspended in fresh medium, and placed on a roller drum in a 37ºC incubator. Cells were maintained in log-phase growth for a 2-day expression period and then cloned in medium containing BBL agar with TFT for selection and without TFT for determination of viability. After 10-13 days of incubation at 37ºC, colonies were counted and colony size distribution was determined using an automatic colony counter modified with a potentiometer. Relative survival values were calculated according to the method described by Clive, D. and J. Spector (1975). Mutat. Res., 31:17-29.

BrdUrd was added to the cultures to be used for the chromosome aberration analysis. The cells were incubated for 14-15 hours with colcemid present for the last 2 hours. Slides were prepared and stained using the fluorescence-plus-Giemsa method and coded. For each concentration, 100 metaphase spreads were analyzed for aberrations. Aberrations were classified as chromatid breaks, deletions, and fragments; triradials, quadriradials, and complex arrangements; chromosome breaks, deletions, fragments, and minutes; and dicentrics, rings, and translocations. Chromatid and chromosome gaps were recorded, but not counted as aberrations. Any metaphase with a chromosome count > or < 46, but within metaphase selection criteria (46±2) was scored as aneuploid.

For the micronucleus assay, cultures were treated with cytochalasin (CYB) and harvested for 12 or 13 hours. Slides were made from cultures corresponding to those used for the aberration analysis. Binucleated cells (1000) were scored for each treatment. Only cells containing 2 separate, well-defined nuclei totally surrounded by cytoplasm were analyzed.

GLP: Unknown

Test Substance: DCA, purity not reported

Results: Mouse lymphoma test: Weakly positive
Cytogenetic test: Positive
Micronucleus test: Negative

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| Remarks: | Mouse lymphoma test: A dose-related cytotoxic and mutagenic effect was observed at concentrations between 100 and 800 µg/mL.<br><br>Cytogenetic test: A positive induction of aberrations was observed at 600 and 800 µg/mL (background level = 8 aberrations/100 cells; 600 µg/mL = 22 aberrations/100 cells; 800 µg/mL = 26 aberrations/100 cells).<br><br>Micronucleus test: There was no significant increased in MN or aneuploidy frequencies. At 600 µg/mL, there was a doubling of the MN frequency over the negative control (background = 5 and 600 µg/mL = 11). But this frequency did not represent a doubling of the historic mean for all negative controls, which was used as an additional criterion for reporting a positive response. |
| Reference: | Harrington-Brock, K. et al. (1998). Mutat. Res., 413(3):265-276. |
| Reliability: | High because a scientifically defensible or guideline method was used. |
| **Type:** | ***In vitro* Clastogenicity Studies** (Mouse Lymphoma Forward Mutation Assay and Chromosome Aberration Study in CHO cells) |
| Cell Type: | Heterozygous L5178Y TK+/- mouse lymphoma cells<br>Chinese hamster ovary (CHO) cells |
| Exogenous Metabolic Activation: | With and without Arochlor 1254-induced rat liver S9 |
| Exposure Concentrations: | Mouse lymphoma test: 0, 125, 500, 2000, 3000, 4000, 5000 µg/mL<br>CHO test: 0, 1250, 2500, 3750, 5000 µg/mL |
| Method: | No specific test guidelines were reported.<br><br>Mouse lymphoma cell forward mutation assay:<br>The assay was initiated with 6 million THMG-treated cells in treatment medium. Test material or vehicle control solutions comprised 10% of the volume. The cells were treated for 4 hours in a shaker, washed twice, and resuspended in growth medium. A preliminary cytotoxicity assay was performed, and doses for the mutation assay were selected based upon cell counts approximately 20 hours later. In the mutagenicity tests, the cells were washed after the treatment phase, resuspended in growth medium and returned to the incubator for a 2-day expression period. At that time, 6 cultures showing an increase in cell density were |

chosen for mutant analysis. Three million cells were seeded onto triplicate dishes with cloning medium containing 5-trifluorothymidine (TFT) in a humidified incubator. Approximately 600 cells were also seeded into cloning medium without TFT for determination of viable colonies. After 10-14 days, colonies were counted with an automatic colony counter equipped with a potentiometer. Mutant frequencies were expressed as the total number of mutant colonies found in each set of 3 cloning dishes to the total number of cells seeded.

Replicate positive and negative (solvent) cultures were included in each experiment. The positive controls included methyl methane sulfonate (MMS) and methylcholanthrene (MCA).

S9 mix was prepared by combining S9, phosphate-buffered saline and a neutralized solution of nicotinamide adenine dinucleotide phosphate (NADP) and isocitrate.

Chromosome aberration assay in CHO cells:
A subculture from seeding 300,000 cells into complete McCoy's medium was made and incubated for 25-26 hours with 5-bromo-2'-deoxyuridine (BD). Cells were then washed, and incubated for 2 hours in fresh medium containing BD and demecolcine (to arrest further divisions). The cells were then harvested, fixed, and stained. Harvests after 10 and 20 hours were identified as useful indicators of the clastogenic effect of BD. Aberrations were analyzed with between 19 and 23 centromeres. For each replicate assay, 100 cells were examined for aberrations including a) simple, including chromatid breaks, chromosome breaks, double dot fragments; b) complex, including interstitial deletions, triradial configurations, quadriradial configurations, complex arrangements, dicentrics, dicentric-plus-fragment, tricentric, quadricentric, pentacentric, and hexacentric. Cells exhibiting more than 10 aberrations were treated statistically as if they had precisely 10 aberrations. Data were collected as counts of aberrations, proportions of cells with aberrations, proportion of cells with more than 1 aberration, and dose-related increases in aberration frequency. Fisher's exact test with an adjustment for multiple comparisons was used.

GLP: Yes

Test Substance: DCA, purity > 99.5%

Results: Mouse lymphoma test: Negative

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| | CHO test: Negative |
| Remarks: | Mouse lymphoma test: Without S9 activation, vehicle controls generated 113±8 mutant colonies and supported 449±59 viable colonies. At all doses studied (125-5000 µg/mL), DCA generated 135±17 mutant colonies and supported 473±32 viable colonies, without any dose-related trend. Cloning frequencies were 99.2-118.5% for all doses of DCA. Mutant frequencies were 41.9-57.9 for the vehicle and 49.4-65.3 for DCA without a dose-related trend.<br><br>With S9 activation, vehicle controls generated 142±11 mutant colonies and supported 499±65 viable colonies. At all doses studied, DCA generated 134±14 mutant colonies and supported 471±22 viable colonies, without any dose-related trend. Cloning frequencies were 89.1-100% for all doses of DCA. Mutant frequencies were 45.7-59.6 for the vehicle and 48.8-61.7 for DCA without a dose-related trend.<br><br>CHO test: The 5000 µg/mL plates were sometimes lost, due to evidence of lethality characterized by floating debris. Neither the 10- nor the 20-hour harvests demonstrated any dose-related trends or evidence of a mutagenic potential for DCA, with or without S9 activation. |
| Reference: | Fox, A. W. et al. (1996). Fundam. Appl. Toxicol., 32:87-95. |
| Reliability: | Medium because a suboptimal study design was used. |

**Additional References for *In vitro* Clastogenicity:**

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Ono, Y. et al. (1991). Suishitsu Odaku Kenkyu, 14(9):633-641.

Chang, L. W. et al. (1992). Environ. Mol. Mutagen., 20:277-288.

Nelson, G. M. et al. (2001). Toxicol. Sci., 60(2):232-241.

Giller, S. et al. (1997). Mutagenesis, 12:321-328.

Harrington-Brock, K. et al. (1992). Environ. Mol. Mutagen., 19(20):24.

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| **Type:** | ***In vivo* Mouse Micronucleus Assay and Single Cell Gel Assay** |
| Species/Strain: | Mice/B6C3F1 |
| Sex/Number: | Male/10 per group |

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| Route of Administration: | Oral via drinking water |
| Concentrations: | Study 1: 0, 0.5, 1, 2, 3.5 g/L<br>Study 2: 0, 3.5 g/L |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. |

DCA was dissolved in deionized water at concentrations of 0.5, 1, 2, and 3.5 g/L. The pH was adjusted to 6.8-7.4 with sodium hydroxide. The solutions were administered to the mice in glass water bottles fitted with Teflon stoppers and double-balled sipper tubes. Fresh drinking water solutions were prepared weekly. Control animals received the vehicle (deionized water).

In study 1, effects of the antioxidant vitamin E on DCA-induced MN and DNA damage was measured by the single cell gel (SCG) assay. Half of the mice in the control and 3.5 g/L groups were injected with 100 mg/kg Vitamin E 2 days prior to the initiation of exposure and at 7 day intervals thereafter. The mice were sacrificed following 9 or 28 days of treatment.

Study 2 was a stop-exposure experiment, in which mice were exposed to either 0 or 3.5 g/L DCA for 31 weeks. At 10 and 26 weeks, groups of mice were removed from the DCA regimen and transferred to deionized water for the remainder of the 31-week study. One group was exposed to 3.5 g/L DCA for the entire 31-week exposure period. All mice were sacrificed at 31 weeks.

At sacrifice, peripheral blood was obtained from the tail vein. For SCG analysis (28-day study only), 1 drop of blood was added to a microcentrifuge tube containing RPMI 1640 media. Two drops of blood were used to prepare peripheral blood smears. When dry, the slides were fixed in absolute methanol. Slides from the 9- and 28-day treatments were stained with acridine orange in sodium phosphate buffer, rinsed, and coverslip mounted. Slides from the 31-week DCA stop-exposure experiment were analyzed for MN frequency, as well as centromere status of the MN, by method of Krishna, G. et al. (1992). Mutat. Res., 282:159-167. Slides were rehydrated. CREST human anti-kinetochore antibody diluted in B/PBS-T was applied to the slides and incubated for 30 minutes at 37ºC in a

humidified chamber. Slides were then washed 2 times in PBS-T, and a fluoresceinated goat anti-human secondary antibody was applied and incubated for 30 minutes at 37ºC. The slides were again washed twice in PBS-T and propidium iodide was applied to the slides and covered with a coverslip.

MN and percent PCE scoring was conducted on coded slides. In the 9-day exposure study, the number of MN-PCE among 1000 PCE was determined for each animal. For the other studies, the numbers of MN-PCE and MN-NCE were determined among 1000-2000 PCE or MCE, respectively, per animal. Bone marrow toxicity was assessed via the number of PCE among 1000 erythrocytes per animal and expressed as % PCE.

The blood samples for the alkaline SCG assay were processed under yellow light as described by Singh, N. P. et al. (1988). Exp. Cell. Res., 175:184-191 as modified in Tice, R. R. et al. (1992). Mutat. Res., 271:101-113.

The incidence of micronucleated erythrocytes among multiple dose groups was statistically analyzed using a one-tailed trend test, based on pooled data incorporating a variance inflation factor to account for excess interanimal variability. Pairwise comparisons of MN data were analyzed using a one-tailed Pearson Chi square test. The percentage of PCE data was analyzed by a one-way analysis of variance (ANOVA) for multiple dose groups or a two-tailed student's t-test for pairwise comparisons. The extent of DNA migration among multiple dose groups was analyzed using a two-tailed trend test. The effect of vitamin E treatment on DCA-induced damage was analyzed using a two-way ANOVA.

GLP: Unknown

Test Substance: DCA, purity = 99%

Results: Positive

Remarks: A small but statistically significant dose-related increase in the frequency of micronucleated polychromatic erythrocytes (PCEs) was observed after subchronic exposure for 9 days. In addition, at the highest dose tested (3.5 g/L), a small but significant increase in the frequency of micronucleated normochromatic erythrocytes (NCEs) was detected following exposure for = 10 weeks. Coadministration of vitamin E did not affect the ability of DCA to induce this damage.

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| | The SCG technique suggested the presence of DNA crosslinking in blood leukocytes in mice exposed to 3.5 g/L DCA for 28 days. |
| Reference: | Fuscoe, J. C. et al. (1996). Environ. Mol. Mutagen., 27(1):1-9.<br><br>Salman, T. et al. (1996). Cancer Mol. Biol., 3(1):725-736. |
| Reliability: | High because a scientifically defensible or guideline method was used. |
| **Type:** | ***In vivo* Mutagenicity in Mouse Liver** |
| Species/Strain: | Mice/B6C3F1 |
| Sex/Number: | Male/ |
| Route of Administration: | Oral via drinking water |
| Concentrations: | 0, 1, 3.5 g/L |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.<br><br>DCA was administered *ad libitum* in deionized drinking water for 4, 10, or 60 weeks. The pH was adjusted to 6.8-7.2 by addition of sodium hydroxide. Control mice were given deionized water *ad libitum*. At each time point, mice were sacrificed. Livers were removed, homogenized in buffer, quick frozen in liquid nitrogen, and stored at -80°C until DNA isolation.<br><br>Genomic DNA was isolated from homogenized liver aliquots by digestion with proteinase K, followed by phenol/chloroform extraction and ethanol precipitation. Because livers from mice exposed for 60 weeks exhibited hypertrophy with glycogen deposition, a modification was made to the procedure. Before ethanol precipitation of the DNA, the upper aqueous phase was collected and dialyzed overnight at room temperature against 1X TE. The next day the 1X TE was changed every 3-4 hours, followed by a final dialysis overnight at room temperature. The dialyzed DNA was collected and stored at 4°C.<br><br>As DNA was needed for packaging, the dialyzed DNA was precipitated with room temperature ethanol. The resulting DNA pellet was resuspended in 1X TE and allowed to stand at room temperature for 24 hours before storage at 4°C. The lambda vector was recovered as phage particles by mixing the genomic DNA with lambda packaging extracts *in vitro*. |

The phage particles were assayed for lacI$^-$ mutations by infecting *E. coli* strain SCS-8 for 15 minutes at 37°C, mixing with prewarmed LB + $MgSO_4$ top agarose containing 5-bromo-4-chloro-3-indolyl-ß-D-galactoside and pouring onto assay trays containing LB + $MgSO_4$ agar. After an overnight incubation at 37°C, plates were examined for the presence of blue mutant plaques on a background of colorless non-mutant plaques. Blue lacI$^-$ plaques were isolated as agarose plugs, eluted into SM buffer, and plated at low density to verify phenotype and purify the mutant clone. Mutant frequencies were expressed as the number of confirmed blue plaques divided by the total number of plaques. The total number of plaques was determined by counting 5 representative square regions of each plate and multiplying the average count by a factor proportional to the total plate area.

Mutants for sequencing were selected from treated and control animals from the 60-week time point. If available, 10 mutants were sequenced from each animal. SM buffer containing resuspended mutant plaque particles was spotted on a LB + $MgSO_4$ agar plate already overlaid with top agarose containing SCS-8 cells and X-gal. The plates were kept at room temperature until dry and then the plate was incubated overnight at 37°C. A portion of the top agarose from the resulting "megaplaque" was placed into a tube with HPLC water and heated for 10 minutes. The boiled preparation was centrifuged to pellet bacterial debris. The supernatant was purified with a PCR product purification kit and then sequenced with a DNA sequencer.

The Cochran-Armitage test was used to determine the statistical significance of increases in mutant frequency. Comparison of mutation spectra was performed using the hypergeometric test.

GLP: Unknown

Test Substance: DCA, purity not reported

Results: Positive

Remarks: At both 4 and 10 weeks of treatment, there was no significant difference in mutant frequency between treated and control groups. At 60 weeks, mice treated with 1.0 and 3.5 g/L DCA showed a 1.3-fold and 2.3-fold increase, respectively, in mutant frequency over controls. The mutation spectrum recovered from mice treated with 3.5 g/L DCA for 60 weeks contained G:C to A:T transitions (32.79%) and G:C to T:A transversions (21.31%). In

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| | contrast, G:C to A:T transitions comprised 53.19% of the recovered mutants among control animals. |
| Reference: | Leavitt, S. A. et al. (1997). Carcinogenesis, 18:2101-2106.<br><br>Leavitt, S. A. and J. A. Ross (1997). Proc. Am. Assoc. Cancer Res., 38:125. |
| Reliability: | High because a scientifically defensible or guideline method was used. |
| **Type:** | ***In vivo* Mouse Micronucleus Assay** |
| Species/Strain: | Rats/Crl:CD (SD) BR |
| Sex/Number: | Male and Female/5 per sex per group |
| Route of Administration: | i.v. |
| Concentrations: | 0, 275, 550, 1100 mg/kg |
| Method: | No specific test guideline was reported; however, the procedures described by Heddle, J. A. et al. (1983). Mutat. Res., 123:61-118 were noted.<br><br>Cyclophosphamide was used as the positive control and was administered on a single day. DCA was administered daily for 3 days. The negative control was 5 mL/kg saline.<br><br>The bone marrow harvest was by sharp dissection of the femurs and excision of the epiphyses. Marrow was flushed into centrifuge tubes containing bovine serum albumin solution. The tissue was pelleted by centrifugation. The supernatant was aspirated and portions of the pellet allowed to dry as a film on microscope slides. Slides were fixed with methanol and stained with acridine orange. Fluorescence microscopy was used for examination of micronucleus formation. Polychromatic to normochromatic erythroid ratios were calculated.<br><br>An overall positive result was defined as a dose-related increase in micronucelated polychromatic erythrocytes or the detection of a reproducible and statistically significant positive response in at least 1 dose group. |
| GLP: | Yes |
| Test Substance: | DCA, purity >99.5% |
| Results: | Negative |
| Remarks: | All animals dosed with 1100 mg/kg were prostrate, or ataxic and languid, after dosing each day. |
| Reference: | Fox, A. W. et al. (1996). Fundam. Appl. Toxicol., 32:87-95. |
| Reliability: | High because a scientifically defensible or guideline method was used. |

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| **Type:** | **8-OH DNA adducts** |
| Species/Strain: | Mice/B6C3F1 |
| Sex/Number: | Male/6 per group |
| Route of Administration: | Oral via drinking water |
| Concentrations: | 0, 0.1, 0.5, 2.0 g/L |
| Method: | No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. |

Mice were administered 0.1 or 3.0 g/L DCA *ad libitum* via drinking water for 3 or 10 weeks. Mice were sacrificed after 21 or 71 days. The livers were excised, weighed, and snap frozen in liquid nitrogen where they were stored at -195ºC until isolation of liver nuclei.

Liver nuclei were isolated by the method of Lynch, W. W. et al. (1970). J. Biol. Chem., 245:3911-3916. Genomic DNA was isolated from purified liver nuclei by a modification of a previously reported method (Marmur, J. (1961). J. Mol. Biol., 3:208-218). Phenol was omitted to avoid artifactual formation of 8-OH-dG.

Analysis of DNA hydrolysates was performed using high performance liquid chromatography with simultaneous UV and electrochemical detection as described by Kasai, H. and S. Nishimura (1984). Nucleic Acids Res., 12:2137-2145 and Floyd, R. A. et al. (1986). Free Radic. Res. Commum., 1:163-172.

Palmitoyl-CoA oxidase activity was measured by the method of Lazarow, P. B. and C. de Duve (1976). Proc. Natl. Acad. Sci. USA, 73:2043-2046.

For laurate hydroxylase activity, differential centrifugation was performed on liver homogenates based on previously described methods in Okita, R. T. and J. O. Okita (1992). Arch. Biochem. Biophys., 294:475-481. Total cytochrome P450 content was measured by the method of Estabrook, R. W. and J. Werringloer (1978). Methods Enzymol., 52:212-220. Cytochrome P450 4A activity was measured using a modified reverse-phase HPLC method using (1-$^{14}$C)-laurate as substrate (Okita, R. T. et al. (1993). J. Biochem. Toxicol., 8:135-144). The modification consisted of extracting once in methylene chloride in place of 3 times

in ethyl acetate.

Experiments were individually assessed across dose using a one-way ANOVA with separation of means being accomplished by Tukey's multiple comparison test.

GLP: Unknown
Test Substance: DCA, purity =99%
Results: Negative
Remarks: Body weights of DCA-treated mice were not affected in either the 3- or 10-week experiment. Dose-related increases in the absolute and relative liver weights were evident for both the 3- and 10-week treatments.

DCA produced a small but significant elevation in the activity of acyl-CoA oxidase only in the 2.0 g/L group in the 3-week experiment. In the 10-week experiment, minor, but statistically significant, increases were observed in the 0.1 and 0.5 g/L groups, but the effect was not observed in the 2.0 g/L group.

DCA produced no significant elevation in the 12-OH/11-OH ratios at concentrations as high as 2.0 g/L.

Levels of 8-OH-dG in the DCA-treated mice were not significantly different from controls in the 3-week or 10-week experiment.

Reference: Parrish, J. M. et al. (1996). Toxicology, 110:103-111.
Reliability: High because a scientifically defensible or guideline method was used.

**Type:** **DNA Strand Breaks**
Species/Strain: Mice/B6C3F1
Sex/Number: Male/5-6 per group
Route of Administration: Oral gavage
Concentrations: 0, 10, 500 mg/kg
Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Experiment 1:
Mice were gavaged with 10 or 500 mg/kg DCA dissolved in 1% aqueous Tween 80. Controls received Tween 80. Mice were sacrificed at 1, 2, 4, 8, and 24 hours after compound administration and the livers were removed. Single strand breaks in hepatic DNA were determined using an alkaline

unwinding assay. The fraction of DNA unwound was calculated.

Experiment 2:
Mice were gavaged with 500 mg/kg DCA as described in experiment 1. Peroxisome proliferation was determined by measuring palmitoyl-CoA oxidation (PCO) in liver homogenates prepared from mice sacrificed at 1, 2, 4, 8, and 24 hours after compound administration. The ability of the liver homogenates to oxidize palmitoyl-CoA oxidation was measured using a radioisotopic method described by Lazarow, P. B. (1981). Methods Enzymol., 72:315.

Experiment 3:
Mice were given 500 mg/kg DCA by gavage for 10 consecutive days. Clofibrate was used as a positive control in this experiment. Mice were sacrificed 24 hours after their last dose and the livers were excised and weighed. The left lateral lobe was used for light and electron microscopy studies, and the remaining liver was used for PCO determinations as described in experiment 2. In the electron microscopy work, the number of peroxisomes present in each sample was recorded.

Statistical comparisons were made using the Student's t-test. PCO data and liver weight data from the repeated dose experiment and electron micrographs were analyzed by ANOVA with Duncan's New Multiple Range test for mean separation.

GLP: Unknown

Test Substance: DCA, purity 99+%

Results: Positive

Remarks: DCA significantly increased the rate of alkaline unwinding of hepatic DNA at 1, 2, and 4 hours after administration. The rate returned to the range of the controls 8 hours after exposure.

No evidence of peroxisomal PCO activity was observed within 24 hours.

Repeated doses of DCA had no effect on the body weight gain of the mice. DCA did significantly increase liver weight, as well as liver to body weight ratio. Repeated doses also induced peroxisomal synthesis as measured by the PCO activity of liver homogenates. Counts of peroxisome profiles were significantly higher in DCA-treated livers

when compared with control livers.

Upon histopathlogic examination, DCA produced a marked cellular hypertrophy uniformly throughout the liver. The hepatocytes were approximately 1.4 times larger in diameter than the control liver cells. An increase in PAS staining was observed indicating glycogen deposition. Multiple white streaks were grossly visible on the surface of the livers. The white areas corresponded with subcapsular foci of coagulative necrosis.

Reference: Nelson, M. A. et al. (1989). Toxicology, 58(3):239-248.

Reliability: High because a scientifically defensible or guideline method was used.

**Additional References for *In vivo* Genetic Toxicity:**

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Nelson, M. A. and R. J. Bull (1988). Toxicol. Appl. Pharm., 94:45-54.

Austin, E. W. et al. (1996). Fundam. Appl. Toxicol., 31:77-82.

Chang, L. W. et al. (1992). Environ. Mol. Mutagen., 20:277-288.