

AR201-13125B

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The studies listed below were selected to represent the best available study design and execution for these HPV toxicity endpoints. Other data of equal or lesser quality are not summarized, but are listed as additional references in this document. Additionally, this summary does not contain references to glycolic acid used in treatment of dermatologic conditions.

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**1.0 Substance Information**

**CAS Number:** 79-14-1

**Chemical Name:** Acetic acid, hydroxy-

**Structural Formula:** HO-CH<sub>2</sub>-COOH

**Other Names:** Glycolic acid  
Hydroxyethanoic acid  
HAA  
Glycollic acid  
Glypure®  
alpha-Hydroxyacetic acid  
Gluc-hydroxy-acid  
2-Hydroxyacetic acid

**Exposure Limits:** 3 ppm (10 mg/m<sup>3</sup>), 8- and 12-hour TWA: DuPont  
Acceptable Exposure Limit (AEL)

**2.0 Physical/Chemical Properties**

**2.1 Melting Point**

**Value:** 78-79°C (solid)

**Decomposition:** No Data

**Sublimation:** No Data

**Method:** No Data

**GLP:** unknown

**Reference:** Sax, N. I. and R. J. Lewis, Sr. (eds.) (1987). Hawley's Condensed Chemical Dictionary, 1<sup>st</sup> ed., p. 620, Van Nostrand Reinhold Co., New York, NY.

**Reliability:** Not assignable because limited study information was available.

**Value:** 10°C (Saturation point of a 70% solution)

**Decomposition:** No Data

**Sublimation:** No Data

**Method:** No Data

**GLP:** Unknown

**Reference:** Sax, N. I. and R. J. Lewis, Sr. (eds.) (1987). Hawley's Condensed Chemical Dictionary, 1<sup>st</sup> ed., p. 620, Van Nostrand Reinhold Co., New York, NY.

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Reliability: Not assignable because limited study information was available.

**Additional References for Melting Point:**

**Solid**

Budavari, S. (ed.) (1996). The Merck Index, 12<sup>th</sup> ed., p. 766, Merck & Co., Inc., Whitehouse Station, NJ,

DuPont Co. (2000). Material Safety Data Sheet No. DUO05926 (March 8).

Hoechst AG (1994). Product Information, Glycolic Acid 57% in Aqueous Solutions, Department of Marketing – Chemicals (1 1/21/94) (cited in IUCLID (1998). IUCLID Data Sheet “Glycolic acid” (October 6)).

Lewis, R. J., Sr. (2000). Sax’s Dangerous Properties of Industrial Materials, 10<sup>th</sup> ed., p. 1882, John Wiley & Sons, Inc., New York.

Verschueren, K. (1983). Handbook of Environmental Data on Organic Chemicals, 2<sup>nd</sup> ed., p. 697, Van Nostrand Reinhold Co., New York.

**70% Solution**

DuPont Co. (1985). Product Information Bulletin: Hydroxyacetic Acid 70% Solution. Technical Properties, Uses, Storage, and Handling.

DuPont Co. (1999). Material Safety Data Sheet No. DUO05927 (December 7).

**57% Solution**

Hoechst AG (1994). Product Information, Glycolic Acid 57% in Aqueous Solutions, Department of Marketing – Chemicals (1 1/2 1194) (cited in IUCLID (1998). IUCLID Data Sheet “Glycolic acid” (October 6)).

Hoechst AG (1996). EC Safety Data Sheet, Glycolic Acid in 57% Aqueous Solution (6/19/96) (cited in IUCLID (1998). IUCLID Data Sheet “Glycolic acid” (October 6)).

2.2 **Boiling Point**

Value: No Data  
Decomposition: Decomposes at 100°C (solid)  
Pressure: No Data  
Method: No Data  
GLP: unknown

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Reference: **Gerhartz, W.** (exec. ed.) (1 P&present). **Ullmann's Encyclopedia of Industrial Chemistry, 5<sup>th</sup> ed.**, Vol. A1, p. 509, VCH Publishers, **Deerfield Beach, FL.**

Reliability: Not assignable because limited study information was available.

Value: **112°C** (70% solution)

Decomposition: No Data

Pressure: 760 mm Hg

Method: No Data

GLP: u n k n o w n

Reference: **DuPont Co.** (1999). Material Safety Data Sheet No. DUO05927 (December 7).

Reliability: Not assignable because limited study information was available.

#### **Additional References for Boiling Point:**

##### **Solid**

Lewis, R. J., Sr. (2000). **Sax's Dangerous Properties of Industrial Materials, 10<sup>th</sup> ed.**, p. 1882, John Wiley & Sons, Inc., New York.

Verschueren, K. (1983). **Handbook of Environmental Data on Organic Chemicals, 2<sup>nd</sup> ed.**, p. 697, Van Nostrand Reinhold Co., New York.

##### **70% Solution**

**DuPont Co.** (1985). product Information Bulletin: Hydroxyacetic Acid 70% Solution. Technical Properties, Uses, Storage, and Handling.

Hoechst AG (1994). Product Information, Glycolic Acid 57% in Aqueous Solutions, Department of Marketing – Chemicals (1 1/21/94) (cited in IUCLID (1998). IUCLID Data Sheet “Glycolic acid” (October 6)).

Hoechst AG (1996). EC Safety Data Sheet, Glycolic Acid in 57% Aqueous Solution (6/19/96) (cited in IUCLID (1998). IUCLID Data Sheet “Glycolic acid” (October 6)).

### **2.3 Density**

Value: 1.36 g/cm<sup>3</sup> (solid)

Temperature: **26°C**

Method: No Data

GLP: unknown

Results: No additional data.

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Reference: DuPont Co. (2000). Material **Safety** Data Sheet No. DU005926 (March 8).  
Reliability: Not assignable because limited study information was available.

Value: 1.25 g/cm<sup>3</sup> (70% solution)  
Temperature: 26°C  
Method: No Data  
GLP: unknown  
Results: No additional data.

Reference: DuPont Co. (1999). Material Safety Data Sheet No. DU005927 (December 7).  
Reliability: Not assignable because limited study information was available.

**Additional References for Density:**

Solid: None Found.

70% Solution

DuPont Co. (1985). Product Information Bulletin: Hydroxyacetic Acid 70% Solution. Technical Properties, Uses, Storage, and Handling.

57% Solution

Hoechst AG (1994). Product Information, Glycolic Acid 57% in Aqueous Solutions, Department of Marketing – Chemicals (1 1/2 1/94) (cited in IUCLID (1998). IUCLID Data Sheet “Glycolic acid” (October 6)).

**Hoechst** AG (1996). EC Safety Data Sheet, Glycolic Acid in 57% Aqueous Solution (6/1 9/96) (cited in IUCLID (1998). IUCLID Data Sheet “Glycolic acid” (October 6)).

% Glycolic Acid Not Specified

Freier (ed.) (1976). Aqueous Solutions: Data for Inorganic and Organic Compounds 1, p. 277, Walter de Gruyter, Berlin, Germany, New York (cited in IUCLID (1998). IUCLID Data Sheet “Glycolic acid” (October 6)).

**Gerhartz**, W. (exec. ed.) (1985 to present). Ullmann's Encyclopedia of Industrial Chemistry, 5<sup>th</sup> ed., Vol. A1, p. VA13 509, VCH Publishers, Deerfield Beach, FL.

**2.4 Vapor Pressure**

Value: 0.017 mm Hg

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Temperature: 25°C  
Decomposition: No Data  
Method: Extrapolated  
GLP: Not Applicable  
Reference: Daubert, T. E. and R. P. Danner (1985). Data Compilation Tables of Properties of Pure Compounds, p. 450, American Institute of Chemical Engineers (SRC Database).

Daubert, T. E. and R. P. Danner (1991). Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation. Supplement 1, Design Institute for Physical Property Data, American Institute of Chemical Engineers, Hemisphere Pub. Corp., New York, NY (SRC Database).  
Reliability: Not assignable because limited study information was available.

Value: Nil (70% solution)  
Temperature: No Data  
Method: No Data  
GLP: unknown  
Reference: DuPont Co. (1999). Material Safety Data Sheet No. DUO05927 (December 7).  
Reliability: Not assignable because limited study information was available.

#### **Additional References for Vapor Pressure:**

##### **Solid**

Daubert, T. E. and R. P. Danner (1989). Thermodynamic Properties of Pure Chemicals Data Compilation, Taylor and Francis, Washington, DC (**HSDB/5227**).

DuPont Co. (2000). Material Safety Data Sheet No. DUO05926 (March 8).

70% Solution: None Found.

##### **57% Solution**

Hoechst AG (1994). Product Information, Glycolic Acid 57% in Aqueous Solutions, Department of Marketing - Chemicals (1 1/21/94) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Hoechst AG (1996). EC Safety Data Sheet, Glycolic Acid in 57% Aqueous Solution (**6/19/96**) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

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## 2.5 Partition Coefficient (log Kow)

Value: -1.11  
Temperature: 19°C  
Method: Measured; estimated accuracy  $\pm 20\%$   
GLP: **Unknown**  
Reference: Collander (1951). Acta Chem. Scand., 5:774-780 (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).  
Reliability: Not assignable because limited study information was available.

### Additional References for Partition Coefficient (log Kow):

Anon. (1996). Epiwin Version 2.0 Syracuse Research Corporation, Environmental Science Center Merrill Lane, Syracuse, NY 132 10 (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Hansch, C. and A. Leo (1987). The Log P Database, Pomona College, Claremont, CA (**HSDB/5527**).

Hoechst AG (1996). Internal Calculation, Dept. SU Environment/Product Safety (**8/27/96**) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Leo, A. J. (1978). Report on the Calculation of **Octanol/Water** Log P Values for Structures in EPA Files (**ISHOW/301724**).

Lewis, R. J., Sr. (2000). Sax's Dangerous Properties of Industrial Materials, 10<sup>th</sup> ed., p. 1882, John Wiley & Sons, Inc., New York.

Verschuere, K. (1983). Handbook of Environmental Data on Organic Chemicals, 2<sup>nd</sup> ed., p. 697, Van Nostrand Reinhold Co., New York.

## 2.6 Water Solubility

Value:, Approximately 2440 g/kg H<sub>2</sub>O (>99% glycolic acid)  
Temperature: 25°C  
pH/pKa: Approximately 1.8 @ 570 g/L  
Method: According to Apelblat and Manzurola (1987). J. Chem. Thermodynamics 19:3 17. original information includes mole fraction 0.3695 (corresponds to about 0.586 mole glycolic acid/mol H<sub>2</sub>O).  
GLP: unknown  
Reference: Hoechst AG (1996). EC Safety Data Sheet, Glycolic Acid

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57% in Aqueous Solution (5/22/1996) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).  
Reliability: Not assignable because limited study information was available.

**Additional References for Water Solubility:**

**Solid**

DuPont Co. (2000). Material Safety Data Sheet No. DUO05926 (March 8).

Budavari, S. (ed.) (1996). The Merck Index, 12<sup>th</sup> ed., p. 766, Merck & Co., Inc., Whitehouse Station, NJ.

Sax, N. I. and R. J. Lewis, Sr. (eds.) (1987). Hawley's Condensed Chemical Dictionary, 1<sup>st</sup> ed., p. 620, Van Nostrand Reinhold Co., New York, NY.

**70% Solution**

DuPont Co. (1999). Material Safety Data Sheet No. DUO05927 (December 7).

Freier (ed.) (1976). Aqueous Solutions: Data for Inorganic and Organic Compounds 1, p. 277, Walter de Gruyter, Berlin, Germany, New York (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Gerhartz (ed.) (1985). Ullmann's Encycloedia of Industrial Chemistry, 5<sup>th</sup> ed., Vol. A13, pp. 507417, VCH Verlagsges.mBH, Weinheim. (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Hoechst AG (1993). EC Safety Data Sheet, Glycolic Acid 70% in Aqueous Solution (5/22/93) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

**% Solution Not Specified**

Van Ness (1978) Kirk-Othmer Encyclopedia of Chemical Technology, 3<sup>rd</sup> ed., Vol. 13, pp. 80-103 (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

**2.7 Flash Point:** Not Applicable.

**2.8 Flammability**

Results: Contact with active metals may produce flammable hydrogen gas (solid)

Method: No Data

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GLP: unknown  
Reference: DuPont Co. (2000). Material Safety Data Sheet No. DUO05926 (March 8).  
Reliability: Not assignable because limited study information was available.

Results: Non-flammable (70% solution)  
Method: No Data  
GLP: unknown  
Reference: DuPont Co. (1999). Material Safety Data Sheet No. DUO05927 (December 7).  
Reliability: Not assignable because limited study information was available.

**Additional References fur Flammability:**

Solid: None Found.

70% Solution

DuPont Co. (1985). Product Information Bulletin: Hydroxyacetic Acid 70% Solution. Technical Properties, Uses, Storage, and Handling.

**3.0 Environmental Fate**

**3.1 Photodegradation**

Concentration: 100 **mmol/L**  
Temperature: 25°C  
Direct Photolysis: Degradation was approximately 20% after 90 minutes. The oxygen consumption was 0.2 **mL/hr**. The reaction was somewhat accelerated catalytically by Fe(III)-ions (1.8 **mL/hr**). Additional presence of the sensitizer anthraquinone-2-sulfonate brought about a further slight acceleration of the oxygen consumption (3.4 **mL/hr**).  
Indirect Photolysis: Not Applicable  
Breakdown  
Products: Formaldehyde was found as a photoproduct.  
Method: Glycolic acid was photodegraded by photolysis in water using a mercury lamp as the light source, calculated light spectrum of 240-300 nm, and concentration of substance of 100 **mmol/L @ 25°C**. The test conditions included reaction volume of 20 **mL** and irradiation in redistilled water in a quartz vessel. The parameter was **O<sub>2</sub>** consumption (determined volumetrically) and analysis of the reaction mixture by capillary isotachophoresis.  
GLP: unknown

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Reference: Klementova and **Wagnerova** (1990). *Mar. Chem.*, **30:89-103**  
(cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).  
Reliability: Medium because a suboptimal study design was used.

### **Additional References for Photodegradation:**

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.

Hoechst AG (1996). Internal Calculation, Dept. SU Environment/Product Safety (8/27/96) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Kolesnikow et al. (1967). *Biokhimiya*, **33:553-556** (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

### **3.2 Stability in Water**

Concentration: Not Applicable  
Half-life: The WVOL program estimates the volatilization half-lives from a model river and lake using the methodology from Lyman et al., 1990, Estimation Handbook (adsorption to suspended solids and sediments is ignored). The user can input an experimental water solubility, vapor pressure, or Henry's Law constant, or EPI will automatically estimate a Henry's Law Constant from SRC's Henry program for this calculation. **WsKow** estimates the water solubility (**WSol**) of an organic compound using the compounds log octanol-water partition coefficient (**Kow**).

The Henry's Law constant for glycolic acid is estimated to be  $8.54 \times 10^{-8} \text{ atm-m}^3/\text{mole}$  (Henry **v3.10** Program, Bond SAR Method in SRC Epiwin **v3.05**) from its estimated vapor pressure, 0.017 mm Hg (Modified Grain Method) and water solubility,  $2.44 \times 10^6 \text{ mg/L}$  (Hoechst AG, 1996). This Henry's Law constant indicates that glycolic acid will not volatilize rapidly from water surfaces. Based on this Henry's Law constant, the estimated volatilization half-life from a model river (1 m deep, flowing 1 **m/sec**, wind velocity of 3 **m/sec**) is approximately 373.8 days (Epiwin **v3.05**). The estimated volatilization half-life from a model lake (1 m deep, flowing 0.05 **m/sec**, wind velocity of 0.5 **m/sec**) is approximately 272 1 days (Epiwin **v3.05**).

% Hydrolyzed: Not Applicable

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Method: Modeled Data: Syracuse Research Corporation EPIWIN v3.05.  
GLP: No  
Reference: Lyman, W. J. et al. (1990). Handbook of Chemical Property Estimation Methods, American Chemical Society.  
  
Hoechst AG (1996). EC Safety Data Sheet, Glycolic Acid 57% in Aqueous Solution (5/22/1996) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).  
  
The following journal article describes the estimation methodology:  
  
Meylan, W. M. et al. (1996). Environ. Toxicol. Chem. **15:100-106**.  
Reliability: Estimated value based on accepted model.

**Additional Reference for Stability in Water:**

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.

Hoechst AG (1996). Internal Calculation, Dept. **SU** Environment/Product Safety (8/27/96) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

**3.3 Transport (Fugacity)**

Media: Air, Water, Soil, Sediments  
Distributions: Air: 0.0018%  
Water: 99.8%  
Soil: 0.0115%  
Sediment: 0.149%  
  
Adsorption  
Coefficient: Not Applicable  
Desorption: Not Applicable  
Volatility: Not Applicable  
Method: Calculated according to Mackay, Level III, Syracuse Research Corporation Epiwin Version 3.05. Emissions (1000 kg/hr) to water compartment using EPA Model Defaults.  
  
Data Used:  
Molecular Weight: 76.05  
Henry's Law Constant:  $8.54 \times 10^{-8}$  atm-m<sup>3</sup>/mole (Henry

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Database)  
Vapor Pressure: 0.017 mm Hg (**Daubert** and Danner, 1985; 1991)  
Log **Kow** : -1 .1 1 (Kowwin program)  
Soil Koc : 0.0318 (Log Kow estimate)  
GLP: Not Applicable  
Reference: Daubert, T. E. and R. P. **Danner** (1985). Data Compilation Tables of Properties of Pure Compounds, p. 450, American Institute of Chemical Engineers (SRC Database).

Daubert, T. E. and R. P. **Danner** (1991). Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation. Supplement 1, Design Institute for Physical Property Data, American Institute of Chemical Engineers, Hemisphere Pub. Corp., New York, NY (SRC Database).

Syracuse Research Corporation EPIWIN v3.05 contains a Level III **fugacity** model. The methodology and programming approach was developed by Dr. Donald Mackay and co-workers which is detailed in:

Mackay, D. (1991). Multimedia Environmental Models: The Fugacity Approach, pp. 67-1 83, 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 value based on accepted model.

#### **Additional Reference for Transport (Fugacity):**

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.

Hoechst AG (1996). Internal Calculation, Dept. SU Environment/Product Safety (8/27/96) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

### **3.4 Biodegradation**

Value: 89.6% **after** 7 days  
Breakdown  
Products: No Data

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Method: The Closed Bottle Test was **performed** to determine if glycolic acid was “readily biodegradable.”\* The Time-O DO (Dissolved Oxygen) was measured and was considered the starting point. The Closed Bottle Test is a closed system, therefore, no additional dissolved oxygen was added to the system for the duration of the test. Biodegradation was measured as the loss of dissolved oxygen within the closed bottle.

Glycolic acid was readily biodegradable, demonstrating 89.6% biodegradability after 7 days. The **28-day** test was terminated after 7 days since glycolic acid demonstrated “ready biodegradability” within the **first** week. The control chemical, sodium acetate, exceeded 60% biodegradability within 14 days, therefore, the test is valid. The Theoretical Oxygen Demand of glycolic acid was calculated as 0.5 mg O<sub>2</sub> per mg of active substance.

GLP: No

References: DuPont Co. (1994). Unpublished Data, **AEM** Laboratory Report No. 142-94.

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

#### **Additional References for Biodegradation:**

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.

Babeu, L. and D. D. Vaishnav (1987). J. Ind. Microbiol., **2:107-115** (also cited in **BIODEG/100610**).

**Billen** et al. (1980). Estuarine Coastal Mar. Sci., **11:279-294** (cited in IUCLID (1998). IUCLID Data Sheet “Glycolic acid” (October 6)).

Heukelekian, H. and M. C. Rand (1955). J. Water Pollut. Contr. Assoc., **27:1040-1053** (also cited in **BIODEG/100611**).

Hoechst AC (1979). Unpublished Data (**10/5/79**) (cited in IUCLID (1998). IUCLID Data Sheet “Glycolic acid” (October 6)).

Howard, P. H. et al. (1992). Environ. Toxicol. Chem., **11(5):593-603**.

Ladd et al. (1982). Appl. Environ. Microbiol., **44:321-329** (cited in IUCLID (1998). IUCLID Data Sheet “Glycolic acid” (October 6)).

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McKinney, R. E. et al. (1956). Sew. Indust. Wastes, 28:547-557.

Pitter and Chudoba (eds.) (1990). Biodegradability of Organic Substances in the Aquatic Environment, p. S. 275, CRC Press (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

### 3.5 **Bioconcentration:**

Value: BCF = 3.162 (log BCF = 0.5). This estimated BCF suggests the potential for bioconcentration in aquatic organisms is low.

Method: Bioconcentration factor (BCF) was calculated by BCFWIN Computer Program, Version 2.13, Syracuse Research Corporation. The estimated value was calculated using a log Kow of -1.11 and a regression-derived equation.

GLP: Not Applicable

Reference: The estimation methodology used by BCFWIN is described in the following document prepared for the U.S. Environmental Protection Agency (OPPT):  
  
"Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient," SRC TR-97-006 (2<sup>nd</sup> Update), July 22, 1997; prepared for Robert S. Boethng, EPA-GPPT, 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., Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212.

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<sub>50</sub>**

Species: *Pimephales promelas* (fathead minnow)

Value: 168 ppm (0.0164%; 95% confidence interval, **0.0154-0.0175%**)

Method: Glass aquaria (20 L) containing 15 L of test solution (19 cm depth) were employed. Ten fish were randomly added to each aquarium (1 aquarium per concentration). Fish were not fed for 48 hours prior to the test, or during the test. Loading was 0.081 g/L. Test solutions were held between 21.9 and 22.7°C (mean 22°C) with aeration and a

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photoperiod of 16 hours light (350 Lux) versus 8 hours darkness. Dissolved oxygen and **pH** were measured in the water control and in all test concentrations before fish were added at the beginning of the test and daily thereafter. Total alkalinity, EDTA hardness, and conductivity of the water control were measured before fish were added at the beginning of the test.

Fathead minnows (aged 103-158 days, mean length 2.1 cm, and mean weight 0.12 g) were exposed to nominal test concentrations of glycolic acid of 0 (water control), 0.0064, **0.0081, 0.010, 0.013, 0.016**, and 0.020% (v/v). Observations were made every 24 hours. No chemical analyses of the test solutions were performed. The **LC<sub>50</sub>** was calculated based on nominal test concentrations using the moving, average-angle method.

GLP: Yes  
Test Substance: Glycolic acid, nominal purity 7090%  
Results: Total mortality was 0, 0, 0, 0, **0, 30**, and 100% at **0, 0.0064, 0.0081, 0.010, 0.013, 0.016**, and **0.020%**, respectively. All deaths occurred within 24 hours. All surviving fish in controls and test solutions behaved normally throughout the test.

At the start of the test, the total **alkalinity** and EDTA hardness of the water control were 81 and 75 **mg/L** as **CaCO<sub>3</sub>**, respectively. The conductivity, dissolved oxygen, and **pH** of the water control at the start of the test were 150 **umhos/cm**, **8.8%**, and 8.1, respectively.

Reference: DuPont Co. (1989). Unpublished Data, Haskell Laboratory Report No. 75 1-89.  
Reliability: High because a scientifically defensible or guideline method was used.

#### **Additional References far 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.

Appiegate, V. C. et al. (1957). Toxicity of 4346 Chemicals to Larval Lampreys and Fishes, United States Department of the **Interior**, Washington, DC.

DuPont Co. (1963). Unpublished Data, Haskell Laboratory Report No. **159-63**.

Data from these additional sources were not **summarized** because insufficient

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study information was available.

Hoechst AG (1988). Unpublished Data, Report No. 88.05 18 (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Mori, Z. (1975). Jan. Kokai, 751121,425 (9/23/75) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Data **from** this additional source was not summarized because the study design was not adequate.

Hidaka et al. (1992). Nippon Suisan Gakkaishi, 58: 1179- 1187 (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

#### 4.2 Acute Toxicity to Invertebrates

**Type:** **48-hour EC<sub>50</sub>**  
**Species:** *Daphnia magna*  
**Value:** 141 mg/L (95% confidence interval, 100300 mg/L)  
**Method:** Nominal concentrations of **25, 50, 100, 200, 400,** and 800 mg/L glycolic acid (not adjusted for purity) and a dilution water control were used in the study. Beakers containing 200 mL of the test solution (6.8 cm test solution depth) were used as test chambers. Four replicate test chambers were used per test concentration with 5 daphnids in each chamber (20 daphnids per concentration). The test chambers were covered with a glass plate during the test. Random numbers were used to assign test concentrations to the test chambers and position of test concentrations in the water bath.

*Daphnia magna* neonates, < 24 hours old were used in the study. Observations of test organisms were made daily. The criterion for the effect (immobility) was the inability to swim at least 2 body lengths in any direction within 15 seconds **after** application of a gentle stimulus. Daphnids were not fed during the test.

A recirculating water bath was used to maintain mean temperature in the test chambers during the **48-hour** test at approximately **20.5°C** with a range of **20.4-20.7°C**. In addition, a continuously recording thermometer was used to check for temperature variation in the water bath. A photoperiod of 16 hours light (approximately 5 1 0-52 1 Lux) and 8 hours darkness was employed, which included 30 minutes of transitional light (7-8 Lux) preceding and

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following the **16-hour** light interval.

Dissolved oxygen concentration, **pH**, and temperature were measured in all replicates of the control and test substance concentrations. These measurements were taken before daphnids were added at test start, and at test end or at total immobility in a replicate chamber. Total alkalinity, EDTA hardness, and conductivity of the water controls and the highest test substance concentration were measured before daphnids were added at the beginning of the test. Test solutions were not aerated during the test.

The **EC<sub>50</sub>** and 95% confidence intervals were calculated by the binomial method.

GLP: Yes  
Test Substance: Glycolic acid (tested as a 70% solution in water), purity **>98%**  
Results: The stock and test solutions were clear and colorless with no insoluble material present. Dilution water quality was acceptable based on OECD and ASTM criteria, with no quantifiable levels of pollutants and pesticides present in the most recent semi-annual dilution water analysis. Total alkalinity for the water control at test start was **54 mg/L CaCO<sub>3</sub>**. EDTA hardness, and conductivity of the dilution water control and **800 mg/L** test solution at test start ranged from **120-123 mg/L CaCO<sub>3</sub>** and **290-490 µmhos/cm**, respectively. The increased conductivity of the **800 mg/L** test concentration can be attributed to the physical properties of the test material. During the test, dissolved oxygen concentrations ranged from **8.8-8.9 mg/L**. The **pH** ranged from **3.0-7.8**. The **pH** decreased as the concentration of test material increased. The mean temperature was **20.5°C** with a range of **20.4-20.7°C**.

No immobility was observed in the dilution water control. Immobility at the end of the study in the **25, 50, 100, 200, 400, and 800 mg/L** nominal test concentrations was **0, 0, 0, 100, 100, and 100%**. There were no sublethal effects observed in the surviving daphnids.

Reference: DuPont Co. (2000). Unpublished Data, Haskell Laboratory Report No. DuPont-3658.  
Reliability: High because a scientifically defensible or guideline method was used.

**Additional References for Acute Toxicity to Invertebrates:** None Found.

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### 4.3 Acute Toxicity to Aquatic Plants

<b>Type:</b>	<b>Growth Rate</b>
Species:	<i>Haematococcus pluvialis</i> (algae)
Value:	No Data
Method:	<i>Haematococcus pluvialis</i> was grown in ASPG medium. To minimize pH changes, 2.0 mM urea was substituted for NaNO <sub>3</sub> as the source of nitrogen without any reduction in the rate of growth. The alga was grown in Erlenmeyer flasks containing medium. Growth was estimated turbidimetrically. Glycolic acid was tested at 5 mM (pH 5.0 and 7.5) for autotrophic growth, and at 10 and 15 mM (pH 5.0 and 7.5) for photoheterotrophic growth (growth in light under essentially CO <sub>2</sub> -free conditions). For autotrophic growth, flasks were plugged with cotton, and incubated at 21 °C under 3000 Lux of continuous illumination provided by cool-white fluorescent lamps for 4 and 8 days. Cultures for photoheterotrophic growth were inoculated directly into Erlenmeyer flasks fitted with screw caps, which were tightened and sealed for 10 days. Cotton plugged flasks for heterotrophic growth were wrapped and incubated at 21°C in the dark for 14 days. Succinate (5.0 mM) was added to maintain the desired pH, and additional controls were established containing 10 mM succinate.
GLP:	unknown
Test Substance:	Glycolic acid, purity not specified
Results:	Glycolic acid caused stimulation of algal growth at pH 7.5 under illumination. When used as a carbon source for heterotrophic growth in the dark, glycolic acid did not support growth during 14 days incubation at pH 5.0 or 7.5. Glycolic acid (pH 5) was an effective substrate for photoheterotrophic growth.
Reference:	McLachlan, J. and J. S. Craigie (1965). <u>Can. J. Bot.</u> , 43: 1449-1456.
Reliability:	Medium because a suboptimal study design was used, as the purity of the test substance was not specified.

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

Data from this additional source was not summarized because the study design was not adequate.

Wright (1970). Symp. Org. Matter Natur. Waters (Univ. Alaska, Sept. 1968), pp. 521-553 (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

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## **5.0 Mammalian Toxicity**

### **5.1 Acute Toxicity**

**Type:** Oral **LD<sub>50</sub>**  
**Species/Strain:** Male and female rats/Crl:CD®(SD)IGS BR  
**Value:** 1938 **mg/kg** (95% confidence interval, 1424-2363 **mg/kg**)  
**Method:** Five male and 5 female rats (aged 57-58 and 78-79-days old, respectively) were intragastrically **intubated** with single doses of **1000, 2000, or 3000 mg/kg** of a 70% solution of glycolic acid. Individual dose volumes for the neat test substance were calculated using fasted body weights obtained prior to dosing, and were based on the test substance density of 1.25 **g/mL**. Additionally, the doses were adjusted for purity. Rats were fasted approximately 18 hours prior to dosing with food being returned to the animals approximately 3 hours after dosing. Rats were dosed at a volume of approximately **1.13, 2.27, and 3.40 mL** per kg of body weight for the **1000, 2000, and 3000 mg/kg** dosage groups, respectively. The test substance was stirred prior to and throughout the dosing procedure.

Observations during the 1 S-day test period included mortality checks, body weight determinations, and observations for clinical signs of toxicity. All rats found dead or sacrificed by design were necropsied to detect grossly observable evidence of organ or tissue damage or dysfunction. The **LD<sub>50</sub>** value for male and female rats was calculated using the method of **Finney**.

**GLP:** Yes  
**Test Substance:** Glycolic acid (tested as a 70% solution in water), purity **>98%**  
**Results:** Mortality ratios for male rats were **0/5, 2/5, and 5/5** at 1000, 2000, and 3000 **mg/kg**, respectively. Mortality ratios for female rats were **0/5, 4/5, and 4/5** at **1000, 2000, and 3000 mg/kg**, respectively. Deaths occurred up to 4 days after dosing. Test substance-related clinical signs most often observed included lethargy (all dose levels, males and females), lung noise (1000 **mg/kg** males and 2000 **mg/kg** males and females), ocular discharge (2000 **mg/kg** males and females), and prostrate posture (2000 and 3000 **mg/kg**, males and females). Other test substance-related clinical signs included hunched-over posture (1000 and 2000 **mg/kg** males), stained face or chin (2000 **mg/kg** males), clear oral discharge (2000 **mg/kg** males), bloating (2000 **mg/kg**

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males), pallor (3000 **mg/kg** males), appeared not to be eating (3000 **mg/kg** males), and moribundity (3000 **mg/kg** males). Weight loss of **3-28%** of the fasted body weight occurred in male rats dosed at 1000 or 2000 **mg/kg**, and in female rats dosed at 2000 or 3000 **mg/kg**. Sporadic weight loss of **2-4%** occurred in some female rats dosed at 1000 or 3000 **mg/kg**.

Test substance-related black stomach discoloration was observed in 5 male rats at the 3000 **mg/kg** dosage level, and 1 and 4 female rats at the 2000 and 3000 **mg/kg** dosage levels, respectively. A black, 1 mm thick layer on the otherwise normal gastric mucosa was easily removed by scraping. Most of the rats with this finding also had stomachs distended with black fluid. The nature of this black material was unknown. Brown lung discoloration, possibly due to a gavage accident, was observed in one female rat at the 2000 **mg/kg** dosage level. The gross observations for the other male and female rats were nonspecific and not indicative of target **organ** toxicity.

Reference: DuPont Co. (1998). Unpublished Data, Haskell Laboratory Report No. DuPont-1614.

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. The studies were not chosen for detailed **summarization** because the data were not substantially additive to the database.

Aldrich (1989). Sigma-Aldrich Material Safety Data Sheets on CD-Rom, Aldrich Chem. Co., Gillingham-Darset, England, originally cited in Chemis (1993). Glycolic acid: Chemis total information, Federal Office of the Environment (search of **12/17/93**) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Althaus, J. (1978). Effective concentrations of health-damaging substances -- Study **from** the Hygiene Institute, **Gelsenkirchen**; Addition of 1978; originally cited in **INFUCHS** (1994). **INFUCHS** Information System (DABAWAS) (Search of **9/1/94**) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Amdur, M. O. et al. (eds.) (1991). **Casarett and Doull's Toxicology, 4<sup>th</sup> ed.**, p. 704.

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**Bove, K. E.** (1966). Am. J. Clin. Path., **45:46-50** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

DuPont Co., Unpublished Data:

(1962a). Haskell Laboratory Report No. 44-62.

(1962b). Haskell Laboratory Report No. 56-62.

(1963). Unpublished Data, Haskell Laboratory Report No. 20-63.

Eastman Kodak Co. (n.d.). Unpublished Data (cited in Patty, F. A. (1963). Industrial Hygiene and Toxicology, 2<sup>nd</sup> revised ed., Interscience Publishers, Inc., New York).

Hoechst AG (1996). EC Safety Data Sheet, Glycolic Acid 57% in Aqueous Solution (6/19/96) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Smyth, H. F. et al. (1941). J. Ind. Hyg. Toxicol., **23(6):259-268**.

Data from these additional sources were not summarized because insufficient study information was available.

Delphaut, J. (195 1). Medicin Tropical, **12:641** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Delphaut, J. (195 1). Medicin Tropical, **12:634-638** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

<b>Type:</b>	<b>Inhalation LC<sub>50</sub></b>
Species/Strain:	Male and female rats/Crl:CD®(SD)IGS BR
Exposure Time:	4 hours
Value:	> 5.2 mg/L (female rats) 3.6 mg/L (male rats)
Method:	One group of 5 male and 5 female rats and 3 groups of 10 male rats each (aged approximately 8 weeks and weighing 237-295 and 198-202 g, respectively) were exposed nose-only to aerosols of glycolic acid (70% solution) for a single 4-hour exposure. These rats were designated for LC <sub>50</sub> determination.

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Male rats were exposed to chamber **concentrations** of either **0.60, 2.1, 3.8, or 5.2 mg/L** glycolic acid. Female rats were exposed to **5.2 mg/L** glycolic acid only. Chamber atmospheres were generated by aerosolization of glycolic acid in air using a nebulizer. The test substance was metered into the nebulizer. Filtered houseline air, introduced at the nebulizer, atomized the test substance and carried the aerosol into the exposure chamber. Chamber concentrations of glycolic acid were controlled by varying the test substance feed rate to the atmosphere generator. The control atmosphere was generated by passing high pressure air through a nebulizer and into the exposure chamber. The atmospheric concentration of glycolic acid was determined by gravimetric analysis at approximately **30- or 45-minute** intervals during each exposure. Two samples to determine particle size distribution were taken during each exposure. Chamber airflow, temperature, relative humidity, and oxygen concentration were recorded.

Because of the dense atmosphere **resulting** from the high aerosol concentration of the test substance, observations for clinical signs could not be conducted during the exposures. During a **14- or 15-day** recovery period, these rats were weighed and observed for clinical signs of toxicity. All surviving rats underwent gross pathological examination at the end of the recovery period. In addition, the nose, larynx, pharynx, and lungs were examined microscopically.

Four groups of 5 male rats each were exposed along with the groups designated for **LC<sub>50</sub>** determination. These rats were designated as satellite animals, and were neither weighed nor observed for clinical signs of toxicity. All surviving satellite animals were sacrificed approximately 24 hours after exposure for microscopic examination of the nose, larynx, pharynx, and lungs. No gross evaluations were conducted on the rats designated as satellite animals.

One group of 10 male rats (designated as controls) was exposed to air only. These animals were neither weighed nor observed for clinical signs of toxicity. Five control rats were sacrificed approximately 24 hours **after** exposure for microscopic examination of the nose, larynx, pharynx, and lungs. The remaining **5** rats were allowed to recover for 14 days, then received the same treatment as the control rats **from** the initial sacrifice. No gross evaluations were conducted on control rats.

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GLP: Yes  
Test Substance: Glycolic acid (tested as a 70% solution in water), purity **>98%**  
Results: Characterization of the chamber atmosphere during each exposure showed the mean total aerosol concentration to be **0.60, 2.1, 3.8, and 5.2 mg/L** glycolic acid. The test atmospheres were considered respirable in rats with a mass median aerodynamic diameter (MMAD) of the aerosol generated during the exposures of 2.3-3.1  $\mu\text{m}$ ; **8.0-18%** of the particles were less than 1  $\mu\text{m}$ , **50-62%** of the particles were less than 3  $\mu\text{m}$ , and **93-99%** of the particles were less than 10  $\mu\text{m}$ . During exposure chamber airflow, temperature, relative humidity, and oxygen concentration were **35 L/min, 20-26°C, 53-69%, and 21%**, respectively.

Mortality ratios in male rats were 0/10, 2/10, 6/10, and **3/5** at **0.60, 2.1, 3.8, and 5.2 mg/L**. The mortality ratio for female rats was **0/5** at 5.2 mg/L (the only concentration tested). Mortality occurred during exposure or within 12 days of exposure. No mortality was observed in male rats designated as satellite animals or in control rats,

Rats **from** the 0.60 and 2.1 mg/L exposure groups experienced slight to severe body-weight losses the day following exposure (**0.3-14%** and **2.4-14%**, respectively). Rats that survived the 3.8 mg/L exposure experienced moderate to severe body-weight losses the day following exposure (**6.5-16%**). Rats exposed to 5.2 mg/L glycolic acid experienced slight to severe body-weight losses the day after exposure (**12-15%** and 0.1-5.1% in male and female rats, respectively). All rats in the **0.60, 2.1, and 3.8 mg/L** groups and one rat in the 5.2 mg/L group experienced an overall weight gain during the recovery period, although some transient weight losses did occur.

Clinical signs of toxicity included lung noise (all exposure levels), gasping (all exposure levels), hunched posture (all exposure levels), ocular and nasal discharge (all exposure levels), stained and/or wet fur and/or perinea (all exposure levels), vocalization (2.1 mg/L), lethargy (2.1 and 3.8 mg/L), sores of the eyes, nose, and/or chin (2.1 and 3.8 mg/L), and alopecia (3.8 mg/L).

No test substance-related target organ gross changes were observed in exposed male or female rats designated for **LC<sub>50</sub>** determination. Test substance-related microscopic changes,

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attributable to tissue irritation, were observed in nose, larynges, and lungs of male rats and nose and larynges of female rats. Minimal to mild nasal lesions, seen in all treated groups, consisted of degeneration/regeneration of respiratory and/or olfactory epithelium.

Mild to severe laryngeal ulceration was present in all treated male groups, and mild effects were present in the female group. Hyperplasia of squamous epithelium, and more rarely, respiratory epithelium of the laryngeal mucosa was noted as a compensatory response to injury in some rats. In addition, hyperplasia of squamous epithelium of the dorsal pharynx was present in some sections in which hyperplasia of squamous epithelium of the larynx was noted.

Minimal to mild subacute/chronic inflammation was present in **lungs** of rats exposed to **2.1, 3.8, and 5.2 mg/L** glycolic acid. In no animal did the change involve all lung lobes. There were no other test substance-related microscopic findings.

Reference: DuPont Co. (1998). Unpublished Data, Haskell Laboratory Report No. DuPont-1516.  
Reliability: High because a scientifically defensible or guideline method was used.

**Additional References for Acute Inhalation Toxicity:**

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

DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 862-81.

Data from this additional source were not summarized because insufficient study information was available.

Eastman Kodak Co. (n.d.). Unpublished Data (cited in Patty, F. A. (1963). Industrial Hygiene and Toxicology, 2<sup>nd</sup> revised ed., Interscience Publishers, Inc., New York).

**Type:** **Dermal Toxicity:** No Data.  
**Type:** **Dermal Irritation**  
**Species/Strain:** Male rabbit/Albino  
**Exposure Time:** 24 hours  
**Method:** A 0.5 mL sample of undiluted material was applied to intact

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and abraded skin under a double 1-inch square of gauze. The treatment area was wrapped for 24 hours. Observations were made at **24, 48,** and 72 hours.

GLP: No  
Test Substance: Glycolic acid (70% technical), purity not specified  
Results: Glycolic acid was classified as a very strong primary skin irritant bordering on corrosive when applied to the skin of one rabbit. It caused strong erythema and mild edema on the intact skin, and strong erythema and necrosis along the lines of abrasion on the abraded skin at 24 hours. All erythema was cleared by 72 hours, however, the necrosis along the line of abrasion remained.  
Reference: DuPont Co. (1962). Unpublished Data, Haskell Laboratory Report No. 44-62.  
Reliability: High because a scientifically defensible or guideline method was used.

**Type: Dermal Irritation**

Species/Strain: Human  
Exposure Time: 30 minutes  
Method: Solutions containing 50% glycolic acid at **pH 1.0** or 70% glycolic acid at **pH 0.6, 1.8, 2.25,** and 2.75 were applied to different areas of the face of 2 elderly subjects with sun-damaged skin and rinsed off 30 minutes later. **After** 48 hours, biopsies were obtained and processed for microscopic examination.

GLP: unknown  
Test Substance: Glycolic acid (50 or 70% solution), purity not specified  
Results: In the case of the 70% solution, there was partial epidermal necrosis and epidermal crusting at **pH 0.6,** epidermal crusting at **pH 1.8,** and partial loss of stratum comeum at **pH 2.25** and 2.75. The site treated with the 50% solution (**pH 1.0**) had lost its stratum comeum, but had no crusting or epidermal necrosis.  
Reference: Becker, F. F. et al. (1996). **Dermatologic Surgery** **22:463-465** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).  
Reliability: Low because an inappropriate method or study design was used.

**Additional References for Dermal Irritation:**

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

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substantially additive to the database.

Hoechst (1984). Unpublished Data, Report No. 84.0024 (cited in IUCLID (1998). ITJCLID Data Sheet "Glycolic acid" (October 6)).

Moy, L. S. et al. (1996). **Dermatologic Surgery**, **22:429-432** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Murad, H. et al. (1995). **Cosmetic Dermatology**, **1:3:285-307** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Data from these additional sources were not summarized because the primary focus of the study was skin corrosion.

**Barrat, M. D. (1996). Toxicology In Vitro, 10:85-94.**

DuPont Co., Unpublished Data:

- (1973). Haskell Laboratory Report No. 418-73.
- (1993a). Haskell Laboratory Report No. 430-93.
- (1993b).** Haskell Laboratory Report No. 425-93.
- (1997a).** Haskell Laboratory Report No. 1997-01084.
- (1997b). Haskell Laboratory Report No. 1997-O 1086.
- (1998). Haskell Laboratory Report No. 1998-01659.
- (1999). Haskell Laboratory Report No. DuPont-3696.

**Hoechst AG (1996). EC Safety Data Sheet, Glycolic Acid in 57% Aqueous Solution (6/19/1996)** (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Data from the following study was not summarized because there was insufficient study information available.

Dermatech (1993). Unpublished Data, submitted by **CTFA (95-AHA-0108)**, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Data from these additional sources were not summarized because the test substance was a mixture or otherwise inappropriate.

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Alfieri, D. R. (1996). Cosmet. Dermatol., **9:42-52** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

**DiNardo, J. C.** (1995). Unpublished Data, submitted by CTFA, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

DiNardo, J. C. (1996). AHAs and Skin Rejuvenation. A Supplement to Cosmetic Dermatology (May), pp. 12-13 (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

DuPont Co. (1994). Unpublished Data, Haskell Laboratory Report No. 56-94.

Hilltop Research, Unpublished Data, submitted by CTFA, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia):

- (1994a). CTFA **95-AHA-0094.**
- (1994b). CTFA **95-AHA-0095.**
- (1995). CTFA **95-AHA-0093.**
- (1996). HTR Reference No. 96-1077-70.

Kostarelos, K. et al. (1999). Cosmet. Toiletries, **114:43-50** (TOXLINE/1999/162867).

**Natura Bisse** (1996). Unpublished Data, submitted by CTFA, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

CTFA, Unpublished Data, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia):

- (1989). CTFA **95-AHA-0027.**
- (1990a). CTFA **95-AHA-0023.**
- (1990b). CTFA **95-AHA-0022.**
- (1990c). CTFA **95-AHA-0028.**
- (1991 a). CTFA **95-AHA-0029.**
- (1991b). CTFA **95-AHA-0066.**

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- (1991c). CTFA **95-AHA-0030**.
- (199%). CTFA **95-AHA-0068**.
- (1992b). CTFA **95-AHA-0065**.
- (1992c). CTFA **95-AHA-0024**.
- (1992d). CTFA **95-AHA-0025**.
- (1993). CTFA **95-AHA-003 1**.
- (1994a). CTFA **95-AHA-0026**.
- (1994b). CTFA **95-AHA-0062**.
- (1995). CTFA **95-AHA-47**.

**Effendy, I. et al. (1995). Acta Dermato-Venereologica, 75:455-458** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

**Kopera, D. et al. (1996). Acta Dermato-Venereologica, 76:461-463** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Milmark Research ( 1994). Unpublished Data, submitted by CTFA (**95-AHA-0001**), Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

**Morganti, P. et al. (1996). J. Appl. Cosmet., 17:79-91** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Thibault, P. K. et al. (1998). **Dermatologic Surgery, 24:573-578** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

TKL Research, Unpublished Data, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia):

- (1994a). CTFA **95-AHA-0106**, TKL Study No. 939253.
- (1994b). CTFA **95-AHA-0100**, TKL Study No. 940101.

Wang, C. M. et al. (1997). **Dermatologic Surgery, 23:23-29** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12,

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National Industrial **Chemicals** Notification and Assessment Scheme,  
Commonwealth of Australia).

Data **from** these additional sources were not summarized because the study design  
was not adequate.

Consumer Product Testing Co., Unpublished Data, submitted by CTFA,  
Washington, DC, Cosmetic Ingredient review (cited in NICNAS (2000). Glycolic  
Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial  
Chemicals Notification and Assessment Scheme, Commonwealth of Australia):

- (1993). CTFA **95-AHA-0104**, Experiment Reference No. C-441-93.
- (1994a). CTFA **95-AHA-0102**.
- (1994b). CTFA **95-AHA-0103**, Experiment Ref. No. **S94-0014-1**.

CTFA, Unpublished Data, Washington, DC, Cosmetic Ingredient Review (cited in  
NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report  
No. 12, National **Industrial** Chemicals Notification and Assessment Scheme,  
Commonwealth of Australia):

- (1994a). CTFA **95-AHA-0048**.
- (1994b). CTFA **95-AHA-0050**.
- (1995). CTFA **95-AHA-46**.

Harrison Research Laboratories, Unpublished Data, submitted by CTFA,  
Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic  
Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial  
Chemicals Notification and Assessment Scheme, Commonwealth of Australia):

- (1994a). CTFA **95-AHA-0101**.
- (1994b). CTFA **95-AHA-0099**.

**DiNardo, J. C.** (1994). Unpublished Data, submitted by CTFA (**95-AHA-0014**),  
Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic  
Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial  
Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Morganti, P. et al (1996). J. Appl. Cosmetol., **17:79-91** (cited in NICNAS (2000).  
Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National  
Industrial Chemicals Notification and Assessment Scheme, Commonwealth of  
Australia).

Hood, H. L. et al. (1999). Food Chem. Toxicol., **37(11):1105-1111**.

Smith, W. P. (1996). J. Cosmet. Sci., **18:75-83** (cited in **NICNAS** (2000).  
Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National

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Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

**Type:** **Dermal Sensitization (Modified Buehler Method)**  
**Species/Strain:** Guinea pigs/ Hartley  
**Method:** The left flank of 20 guinea pigs (weighing 341-516 g on the day prior to the first induction application or on the day of the **range-finding** application) were closely clipped on the day prior to test substance administration (Day -1). The clipping was repeated weekly during the induction phase of the study. The right flank of each *animal* exposed to the test substance in the induction phase was closely clipped on the day prior to the challenge application. Similar areas were clipped on the **left flank (caudal)** and right flank of each animal (exposed to vehicle in the induction phase) on the day prior to challenge applications.

Induction applications were performed using a 26% **w/v** solution of glycolic acid in normal saline solution, and the challenge applications were performed using a 20% **w/v** solution of glycolic acid in normal saline solution. A 0.5 **mL** aliquot of a 26% **w/v** solution of glycolic acid in normal saline solution was applied to the test site, and covered with a piece of occlusive dental dam. Plastic wrap was then wrapped snugly around the trunk of the animal and overwrapped with elastic bandage. Approximately 6 hours after application, the entire wrapping was removed, and the test sites were wiped with normal saline solution and then deionized water.

A separate group of 10 animals (vehicle and test substance irritation control group) were clipped and treated with 0.5 **mL** normal saline solution in the same manner as the test animals.

The test sites were scored for irritation at approximately 24 and 48 hours following application. This procedure was performed at **7-day** intervals for 3 consecutive weeks.

Following the **3<sup>rd</sup>** application of test substance to the test sites, the animals were rested for approximately 15 days. **On** Day 29, the animals were clipped as described above. The challenge application was performed on the right flank using a 0.5 **mL** aliquot of a 20% **w/v** solution of glycolic acid in normal saline solution. Similarly, animals in the vehicle and test substance irritation control group were treated with

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normal saline solution on the left flank and a 0.5 mL aliquot of a 20% w/v solution of glycolic acid in normal saline solution on the right flank. The application of test substance and vehicle were as described above. Approximately 6 hours after application of the test substance and/or vehicle, the entire wrapping was removed, and the test site(s) wiped with normal saline solution and then deionized water. Approximately 24 and 48 hours after the challenge application, the test sites were examined for dermal irritation or signs of elicited sensitization.

A positive control was not run concurrently with the test substance. However, a dermal sensitization test using a positive control (a-hexylcinnamaldehyde, technical grade 85%) was periodically performed to validate the system.

Clinical observations and body weights were recorded.  
Yes

**GLP:**

**Test Substance:**

Glycolic acid (tested as a 70% solution in water), purity >98%

**Results:**

Observations of no to faint redness were noted for guinea pigs exposed to glycolic acid 24 and 48 hours after the 1<sup>st</sup> induction application. No to faint redness at 24 hours, and no redness at 48 hours were observed following the 2<sup>nd</sup> induction application. Scratches at the test site were observed in 8 guinea pigs at 24 hours. Five of these guinea pigs exhibited focal ulcerations and 2 exhibited focal necrosis at 48 hours. Six other animals exhibited exfoliation at 48 hours. No to moderate redness was observed at 24 and 48 hours following the 3<sup>rd</sup> induction. Two incidences of scratches at the test site and 1 incidence of exfoliation were observed at 24 and 48 hours.

After the challenge application, no redness was observed at the test substance sites at 24- and 48-hours. At 48 hours there were 4 incidences of scratches at the test site. The incidence of sensitization was 0/20 (0%).

Dermal irritation was not observed after the induction applications to the vehicle control animals. Challenge application at the vehicle sites also failed to induce dermal irritation. No redness was observed at the test substance irritation control sites 24 and 48 hours after the challenge application.

All vehicle and test substance animals appeared to be normal

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throughout the study. One guinea pig exhibited a weight loss **from** day 14 to day 21. All guinea pigs exhibited an overall gain in body weight during the study. There were no statistically significant differences in individual body weight percentage gains of the test substance group when compared to the vehicle and test substance irritation control group.

Signs of irritation were observed in the animals treated with 2.0%  **$\alpha$ -hexylcinnamaldehyde** in ethanol (HCA; positive control material). The incidence of sensitization after the challenge application of HCA was 50% (**10/20**), and the severity was 0.63 at 24 hours and 0.68 at 48 hours.

It was concluded that repeated administration of glycolic acid did not produce a delayed contact sensitization response to guinea pigs, and is not considered a dermal sensitizer under the study conditions utilized.

Reference: DuPont Co. (1998). Unpublished Data, Haskell Laboratory Report No. DuPont- 1152.  
Reliability: High because a scientifically defensible or guideline method was used.

#### **Additional References for Dermal Sensitization:**

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

AMA Laboratories, Unpublished Data, submitted by CTFA, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). **Glycolic Acid**: priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia):

- (1993a). CTFA **95-AHA-0002**.
- (1993b). CTFA **95-AHA-0005**.
- (1993c). CTFA **95-AHA-0003**.
- (1994). CTFA **95-AHA-0004**.

Consumer Product Testing Co. (1993). Unpublished Data, submitted by CTFA (**95-AHA-0105**), Experiment Ref. No. C-439-93, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

CTFA (1995). Unpublished Data, submitted by CTFA (**95-AHA-49**), Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic

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Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Essex Testing Clinic, Unpublished Data, submitted by CTFA, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia):

- (1994a). CTFA **95-AHA-0089**.
- (1994b). CTFA **95-AHA-0090**.
- (1994c). CTFA **95-AHA-0091**.
- (1994d). CTFA **95-AHA-0092**.
- (1994e). CTFA **95-AHA-0085**.
- (1994f). CTFA **95-AHA-0087**.
- (1994g). CTFA **95-AHA-0086**.
- (1994h). CTFA **95-AHA-0088**.
- (1994i). CTFA **95-AHA-0098**.

Kanengiser, B. E. et al. (1994). Final Report **CRL37594-5**, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Kanengiser, B. E. et al. (1994). Final Report **CRL37594-6**, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Eastman Kodak Co. (**n.d.**). Unpublished Data (cited in Patty, F. A. (1963). Industrial Hygiene and Toxicology, 2<sup>nd</sup> revised ed., Interscience Publishers, Inc., New York).

Goh, C. L. and S. K. Ng (1987). Contact Dermatitis, **17:89-91**.

Data **from** these additional sources were not summarized because insufficient study information was available.

Unilever (1994). Unpublished Data, submitted by CTFA, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Yu, R. J. and E. J. Van Scott (1996). Cosmet. Dermatol., **9:54-62** (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

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Data from this additional source was not **summarized** because the result was inconsistent with the majority of the other findings.

Recherche e **Technologie** Cosmetologique (1996). Unpublished Data, **submitted to CIR**, Study No. **RTC/001/V**, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

<b>Type:</b>	<b>Eye Irritation</b>
Species&train:	Rabbits/Albino
Method:	One-tenth milliliter of the undiluted liquid was placed into the right conjunctival sac of each of 2 albino rabbit eyes. After 20 seconds, 1 treated eye was washed with tap water for 1 minute. The treated eye of the other rabbit was not washed. Observations of the cornea, iris, and <b>conjunctiva</b> were made with a hand-slit lamp at 1 and 4 hours, and at <b>1, 2, 3, 7,</b> and 14 days. <b>Fluor-I-strip</b> <sup>®</sup> stain and a biomicroscope were used at examinations after the day of treatment.
GLP:	No
Test Substance:	Glycolic acid, purity 64%
Results:	Glycolic acid (0.1 <b>mL</b> , undiluted) <b>was</b> corrosive to <b>rabbit</b> eyes. Ocular effects in both the washed and unwashed eye were severe and irreversible. By 14 days, observation was terminated because 1 treated, unwashed eye, which had become very small, had no reaction to light. The other eye, which had been washed following treatment, reacted to light, although the cornea appeared as if it would rupture.
Reference:	DuPont Co. (1977). Unpublished Data, Haskell Report No. <b>446-77</b> .
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. The studies were not chosen for detailed **summarization** because the data were not substantially additive to the database.

DuPont Co. (1964). Unpublished Data, Haskell Laboratory Report No. 33-64.

Carpenter, C. P. and H. F. Smyth, Jr. (1946). Am. J. Ophthalmol., **29(11):1363-1372**.

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Hoechst AG (1984). Unpublished Data, Report No. 84.0037 (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic **acid**" (October 6)).

Hoechst AG (1996). EC Safety Data Sheet, Glycolic Acid 57% in Aqueous Solution (**6/19/1996**) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

McLaughlin, R. S. (1946). Am. J. Ouhthamol., **29(11):1363-1372**.

Data from this addition&l source was not summarized because **insufficient** information was available.

DuPont Co. (1968). Unpublished Data (September 29).

Data from these additional sources were not summarized because the test substance was a mixture or otherwise inappropriate.

Avon Products, Inc. (1995). Unpublished Data, submitted by CTFA (**95-AHA-0052**), Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Tox Monitor Laboratories, Unpublished Data, Washington, DC (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia):

- (1994a). CTFA Number **95-AHA-0076**, TM Study No. **94-28 1**.
- (1994b). CTFA Number **95-AHA-0077**, TM Study No. 94-282.
- (1994c). CTFA Number **95-AHA-0082**, TM Study No. 94-283A.
- (1994d). CTFA Number **95-AHA-0083**, TM Study No. 94-284A.
- (1994e). CTFA Number **95-AHA-0073**, TM Study No. 94-275.
- (1994f). CTFA Number **95-AHA-008 1**, TM Study No. **94-276A**.
- (1994g). CTFA Number **95-AHA-0079**, TM Study No. 94-277.
- (1994h). CTFA Number **95-AHA-0084**, TM Study No. **94-278A**.
- (1994i). CTFA Number **95-AHA-007 1**, TM Study No. 94-377.

Data from this additional source was not summarized because the study design was not adequate.

DuPont Co. (1940). Unpublished Data, Haskell Laboratory Report No. **2-40**.

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## 5.2 Repeated Dose Toxicity

### Study No. 1

**Type:** **90-Day Gavage**  
**Species/Strain:** **Rats/Crl:CD®(SD)IGS BR**  
**Sex/Number:** Male and female/ 40 per sex per dose group  
**Exposure Period:** 90 days  
**Frequency of Treatment:** Daily  
**Exposure Levels:** **0, 150, 300, 600 mg/kg**  
**Method:** Rats (approximately 48 days old) were administered glycolic acid in water via intragastric intubation. Control animals were dosed with water only. Dosing solutions were stored refrigerated until used. Dosing solutions were analyzed 5 times throughout the study. At the 1<sup>st</sup> time point, samples were analyzed to determine concentration and stability. At the remaining time points, samples were analyzed for concentration verification.

Each dosage group was divided into subchronic toxicity, immunotoxicity, neurotoxicity, and reproductive toxicity subsets (10 animals/sex/subset/concentration). Body weights, clinical observations, and individual food consumption were recorded. Ophthalmoscopic examinations were conducted on all rats prior to the start of the study and on surviving subchronic toxicity rats just prior to scheduled sacrifice.

At approximately **midstudy** and near the end of the study, blood was collected from each rat designated as subchronic toxicity animals. Fifteen hematologic parameters and 17 clinical chemistry parameters were measured or calculated. On the day prior to each bleeding time, an overnight urine specimen was collected and 12 urine chemistry parameters were measured or calculated. The rats designated for subchronic toxicity evaluations were sacrificed and necropsied at the end of the study. Each rat was given a gross examination, the weights of 6 organs were recorded, and representative samples of 53 tissues were saved for histopathologic examination. All tissues collected (except nasal tissue without gross lesions) from all animals in the control and 600 **mg/kg** groups were microscopically examined. Liver, kidneys, lungs, and most gross lesions were examined from rats in the intermediate concentration groups.

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After 28 days of glycolic acid administration, **humoral** immune function was evaluated in animals in the immunotoxicity subset. On test day 23, animals designated for immunotoxicity evaluation were injected intravenously with sheep red blood cells (SRBC). Six days after injection, the animals were euthanatized. Following sacrifice, the spleen and thymus were removed and weighed, and serum was collected **from** each rat and analyzed for SRB-specific **IgM** antibody. Sera previously collected from rats injected with **SRBC** and dosed with the known immunosuppressive agent cyclophosphamide served as a positive control.

Rats designated for **neurotoxicity** evaluation underwent functional observational battery (FOB) assessments (encompassing 34 endpoints) and motor activity (MA) evaluations (encompassing 2 dependent variables) once prior to study start, then near the beginning, middle, and end of the study. Six animals per group were evaluated for neuropathology at the end of the study. Six unexposed control animals per sex were randomly selected as negative controls. Rats were euthanatized followed by whole body perfusion fixation, and 17 tissues were saved. Only tissues from the control and 600 **mg/kg** group were microscopically examined.

On test day 97, animals of the reproductive toxicity subset were bred within their respective treatment groups and allowed to deliver and rear their offspring until weaning (postpartum day 21). All parental rats were sacrificed and received a gross pathological examination. Additional details for reproductive and **pup/weanling** information can be found in Section 5.4.

GLP: Yes  
Test Substance: Glycolic acid (tested as a 70% solution in water), purity >98%  
Results: Concentration verification of the samples from the 1<sup>st</sup> time point were within acceptable limits ranging from **95.0-109.3%** of nominal. The mean measured values were **98.7%, 98.3%**, and 107.5% of nominal at 150,300, and 600 **mg/kg**. Measured values for stability were **97.7-109.3%** of nominal concentrations. These data indicated that the test substance was stable in water at room temperature and refrigerated storage conditions; therefore the dosing solutions were considered stable under the conditions of use in this study. Analytical results verifying concentration at the remaining sampling time points showed that percent

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nominal concentrations ranged **from 92.0-102.7%**. The results were within expected variability of the analytical method. These data, together with the concentration verification from the **1<sup>st</sup>** time point, indicate that the test substance was present at the targeted dosing concentrations.

Two compound-related deaths occurred in males at **600 mg/kg**. No other compound-related mortality was observed in this study. No effects in body weight, food consumption, or clinical signs were observed in animals dosed with **150 mg/kg** glycolic acid. Administration of glycolic acid doses of **300 and 600 mg/kg/day** decreased mean body weight, overall body weight gain, food consumption, and food efficiency when compared to their respective controls. The body weight effects observed **in the 600 mg/kg/day** groups were considered adverse, however, the magnitude of the body weight effect observed at **300 mg/kg** was mild, and was not considered adverse. There were no adverse clinical signs indicative of systemic toxicity and no test substance-related ophthalmologic findings observed during this study.

*Subchronic Toxicity Subset:* Toxicologically significant changes in hematologic measurements were increased neutrophils in male rats dosed with **300 (transient) or 600 mg/kg**. Toxicologically significant changes in clinical chemistry and urinalysis parameters included increased urea nitrogen, phosphorus, and **creatinine**, and decreased urine concentration at the end of the study in male rats dosed with **300 (transient) or 600 mg/kg**. All other changes were either not compound-related or considered not to be adverse.

Mean kidney weight (absolute and relative to body weight) of male rats in the **300 and 600 mg/kg** groups was significantly higher. Those weights and gross findings of renal pelvis dilatation correlated with microscopic findings of oxalate crystal nephrosis and unilateral hydronephrosis in the **300 and 600 mg/kg** groups. In addition, hyperplasia of the transitional epithelium of the renal pelvis was observed microscopically in males of the **300 and 600 mg/kg** groups and was considered secondary to the mucosal irritation created by passage of oxalate crystals from the kidney. There were no organ weight, gross, or microscopic **findings** indicative of systemic toxicity observed in female rats treated with glycolic acid. A number of microscopic **findings** in the upper airways and lungs of male and female

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rats in all treated groups were considered irritant effects occurring secondary to aspiration (and/or off-gassing) of the acidic gavage material. No other test substance-related changes were noted.

***Immunotoxicity:*** There were no toxicological changes in the immunotoxicity parameters measured.

***Neurotoxicity:*** There were no toxicological changes in the behavioral parameters of neurotoxicity measured. There were no compound-related gross lesions or microscopic findings observed in the tissues of the nervous system or skeletal muscle examined.

***Reproduction:*** There were no toxicological changes in the measures of reproductive function. There were no changes in organ weights or gross pathology of the reproductive system. There were compound-related gross lesions (dilatation of the pelvis, calculus, chronic progressive nephropathy, and pale discoloration) in the kidneys of male rats in the 600 **mg/kg/day** group that correlated microscopically with oxalate crystal nephropathy similar to that diagnosed in the subchronic toxicity animals. No compound-related gross observations were noted in the **P<sub>1</sub>** females or the **F<sub>1</sub>** weanlings. Additional details for the reproductive toxicity subset can be found in Section 5.4.

Reference: DuPont Co. (1999). Unpublished Data, Haskell Laboratory Report No. DuPont- 1597.

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

**Study No. 2**

**Type:**

Species/Strain:

Sex/Number:

Exposure Period:

Frequency of

Treatment:

Exposure Levels:

Method:

**2-Week Inhalation**

**Rats/Crl:CD<sup>®</sup>**

Male/ 10 per group

2 weeks; **14-day** recovery

6 hours/day, 5 days/week

**0.16, 0.51, 1.4 mg/L**

Rats (7-8 weeks old, weighing 237-263 g) were exposed nose-only to glycolic acid 6 hours/day, 5 days/week for 2 weeks. Aerosol atmospheres of glycolic acid were generated into 20-L cylindrical glass exposure chambers using a **nebulizer**. A small liquid flow was aerosolized off a spherical glass bead by a high-pressure airstream. The aerosol was mixed with dilution air and introduced directly

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into the glass exposure chamber. Chamber atmospheric concentrations were determined by gravimetric **analysis**. Particle size was conducted on the **1<sup>st</sup>** exposure day of the study using a cascade impactor. Chamber temperature was monitored during each exposure.

Body weights and clinical signs were recorded. Clinical laboratory measurements were performed on 10 rats/group following 10 exposures (8 for the 1.4 **mg/L** group) and on 5 rats/group following the **14-day** recovery period. Individual urine samples were collected overnight following the **8<sup>th</sup>** exposure in the high level (1.4 **mg/L**) and the **9<sup>th</sup>** exposure in the remaining levels, and 12 urine chemistry parameters were measured or calculated. A similar urine sample was collected and evaluated **from** animals at all levels at the end of the recovery period.

On the morning after the urine collection, blood was collected from each rat. Fifteen hematologic parameters and 7 clinical chemistry parameters were measured or calculated.

**After** the **8<sup>th</sup>** exposure, 4 rats from the 1.4 **mg/L** exposure level were sacrificed and given a gross examination. Weights of **7** organs were recorded, and 24 tissues were examined microscopically. After the **1<sup>0<sup>th</sup></sup>** exposure, 5 rats from the **0, 0.16, and 0.5 1 mg/L** exposure levels were similarly examined. Remaining rats were sacrificed at the end of the recovery period for similar examination.

GLP:

Yes

Test Substance:

Glycolic acid (tested as a 70% solution in water), purity **>98%**

Results:

The generation of glycolic acid aerosol was such that the actual mean concentrations attained were **0.16, 0.5 1, and 1.4 mg/L** over the entire experiment. These concentrations were presented for glycolic acid; the material tested was 29% water so that the actual concentrations of the material tested were close to the design concentrations of **0.23, 0.72, and 2.0 mg/L**. **In** all of the particle size **determinations**, over 95% of the particles were less than 10 **µm** (mean between 1.5-2 **µm**). Chamber temperatures stayed within **23 ± 2°C** in all groups during the experiment.

All rats survived in the control and 0.16 **mg/L** groups. **One** rat exposed to 0.5 1 **mg/L** was sacrificed in moribund condition. Seven rats exposed to 1.4 **mg/L** glycolic acid were sacrificed *in extremis*. Body weights of rats exposed to

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0.16 **mg/L** glycolic acid were comparable to controls. Statistically significant and severe weight loss were observed throughout the exposure and recovery periods in rats exposed to 0.5 1 **and** 1.4 **mg/L** glycolic acid. **Clinical signs** of toxicity included labored breathing (0.51 and 1.4 **mg/L**), lung noise (0.5 1 and 1.4 **mg/L**), ruffled and discolored **fur** (0.5 1 and 1.4 **mg/L**), red **and** clear nasal and ocular discharges (0.5 1 and 1.4 **mg/L**), poor muscle tone (0.5 1 and 1.4 **mg/L**), and pallor (1.4 **mg/L**).

At the end of the exposure period there were no test substance-related clinical pathology **changes** in rats exposed to 0.16 **mg/L** of the test substance. Rats exposed to 0.5 1 **mg/L** had increased serum activities of aspartate aminotransferase **and** decreased urine volume. These changes had not resolved by the end of the recovery period **in** this group due to 2 of the rats having elevated **alanine** aminotransferase and aspartate aminotransferase levels. Rats exposed to 1.4 **mg/L** had decreased concentrations of serum protein, increased serum activities of **alanine** aminotransferase and aspartate aminotransferase, and decreases in urine volume and **pH**. These changes were interpreted as evidence of a test substance-related effect on the integrity and function of hepatic tissue. These effects were reversible following 14 days of recovery. No other biologically significant changes were observed.

Gross pathological **examination** following the exposure period revealed no test substance-related changes in rats exposed to 0.16 or 0.5 1 **mg/L**. In rats exposed to 1.4 **mg/L**, distended gastrointestinal (GI) tract, small spleen, and small thymus were observed. No effects were observed in rats **after** the recovery period. Microscopically, a very mild diffuse hepatocellular degeneration (eosinophilia and shrinkage) was detected in 1/10 (0/5 following exposure, 1/5 following recovery), 9/10 (5/5 following exposure, 4/5 following recovery), **and** 7/10 (at sacrifice) rats exposed to 0.16, 0.5 1, and 1.4 **mg/L**, respectively. Atrophy and degeneration of the thymus were noted in 5/10 rats exposed to 0.5 1 **mg/L** (2/5 following exposure and 3/5 at the end of recovery) and 8/10 rats exposed to 1.4 **mg/L** (at sacrifice). No other test substance-related microscopic findings were observed.

A comparison of organ and body weights between test and control rats showed no changes in rats exposed to 0.16 **mg/L**.

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Absolute liver, spleen, kidney, and thymus weights were significantly lower following the exposure period in rats exposed to 0.51 and 1.4 **mg/L**. On an organ/body weight basis, rats exposed to 0.16 **mg/L** had an elevated mean lung weight ratio. Rats exposed to 1.4 **mg/L** had elevated organ/body weight ratios for the heart, lung, kidney, and testis, and a decreased **thymus/body** weight ratio. At the end of the recovery period, rats exposed to 0.5 1 **mg/L** had significantly lower lung, liver, kidney, and thymus weights. Rats exposed to 1.4 **mg/L** had significantly lower kidney weights. On an organ/body weight basis, the only difference noted was an increased testis/body weight ratio in rats exposed to 0.5 1 **mg/L**.

The only effect **seen** at the low level was a very mild, **diffuse** hepatocellular degeneration in 1/10 rats 14 days post-exposure, and 0.16 **mg/L** can be considered for all practical purposes a no adverse effect level.

References: DuPont Co. (1983). Unpublished Data, Haskell Laboratory Report No. 114-83.

DuPont Co. (1982). Unpublished Data, Haskell Pathology Report No. 25-82.

Reliability: Kennedy, G. L. and B. A. Burgess (1997). Inhal. Tox., **9(5):435-447**.  
High because a scientifically defensible or guideline method was used.

#### **Additional References for Repeated Dose Toxicity:**

##### **Oral**

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.

**Krop, S. et al. (1945). J. Am. Pharm. Assoc., 34:86-89.**

Richardson, D. E. (1965). Toxicol. Appl. Pharm., 7:507-5 15.

Rose and Carter (1943). Toxicity Study of Glycolic Acid, Central **Research** Laboratories, General Food Corporation, **Hoboken, NJ** (March 29).

Silbergeld, S. and H. E. Carter (1959). Arch. Biochem. Biophys., 84:183-187.

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Selvarn et al. (1992). Pharmacol. Res., **26:385-394** (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Silbergeld, S. (1960). Toxicol. Appl. Pharm., **2:220-224**.

Data from these additional sources were not summarized because the test substance was a mixture or otherwise inappropriate.

DuPont Co. (1940). Unpublished Data, Haskell Laboratory Report No. 1-40.

Ogawa et al. (1986). Hinyokika Kiyo, **32(8):1127-1 133** (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Ogawa et al. (1986). J. Ural., **135:1057-1060** (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Ogawa et al. (1987). Hinyokika Kiyo, **33:1772-1777** (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Data from this additional **source** was not summarized because **insufficient** study information was available.

Sanchez, I. M. and R. J. Bull (1990). Toxicology, **64:33-46**.

### Dermal

Data **from** these additional sources were not summarized because the study design was not adequate.

Argus Research Laboratories, Inc. (2000). Correspondence **from** D. B. Learn to L. Loretz (CTFA), RE: Protocol 1203-005.

Yu, R. J. and E. J. van Scott (1975). U. S. Patent No. 3879537 (April 22) (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

## 5.3 Developmental Toxicity

### Study No. 1

Type:	<b><i>In vivo</i> Developmental Toxicity</b>
Species/Strain:	<b>Rats/Crl:CD<sup>®</sup>BR</b>
Sex/Number:	Females/25 per dose level
Route of Administration:	Gavage
Exposure Period:	Day 7-2 1 of gestation; Cesarean section on Day 22
Frequency of Treatment:	Once/day

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Exposure Levels: **0, 75, 150, 300, 600 mg/kg**  
Method: Solutions of the test material in deionized water were prepared weekly during the study and stored in the refrigerator. Sufficient amounts of solution needed for dose administration were removed **from** the refrigerated containers daily. Samples of each test solution were taken 3 times during the study. Analysis of the first sampling addressed concentration and stability. For the **2<sup>nd</sup>** and **3<sup>rd</sup>** samplings, analyses addressed concentration.

Females were cohabited with males (1: 1) until copulation was confirmed by the presence of a copulation plug in the vagina or on the cageboard. Checks for copulation plugs were made each morning; the day copulation was confirmed was designated as Day 1 of gestation (Day **1G**). Before dosing began, females were randomly assigned to control or experimental groups based on their body weight.

Body weights, clinical signs, and **food** consumption were recorded. Females were euthanized on Day **22G**, and the organs of the thoracic and abdominal cavities were examined for gross pathologic changes. The uterus was removed, weighed, and opened. The types of implants (live and dead fetuses, and resorptions) were counted and their relative positions were recorded. Then the empty uterus was weighed to permit calculation of body weight minus the products of conception. The ovaries were removed and the corpora **lutea** were counted and recorded. The uterus of each apparently nonpregnant rat was opened and stained to detect very early resorptions.

Live fetuses were weighed, sexed, and examined for external alterations. The first live fetus and thereafter every other fetus in each litter was decapitated and examined for visceral alterations and the sex verified. The heads were fixed and examined. The remaining fetuses were euthanized. All fetuses were fixed, stained, and examined for skeletal alterations.

GLP: Yes  
Test Substance: Glycolic acid, purity 99.6%  
Results: Measured concentrations of glycolic acid in dosing formulations at the **1<sup>st</sup>** sampling time point (fresh sample) ranged from **98.6-103%** of nominal. These data indicate that the test substance was at an acceptable level in all formulations. Measured concentrations of glycolic acid in dosing formulations at the **1<sup>st</sup>** sampling point, refrigerated for

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7 days, and then analyzed were **from 95.7-99.8%** of nominal. These data indicate that the test substance was stable for the storage conditions of the study. Measured concentrations for the 2<sup>nd</sup> and 3<sup>rd</sup> sampling points were 99.8-103 and **97.5-102%** of nominal, respectively. These data indicate that the test substance was at an acceptable level in all formulations.

Pregnancy ratios were **25/25, 25/25, 24/25, 23/25**, and **23/25** at **0, 75, 150, 300, and 600 mg/kg**, respectively. There were no mortalities observed at any dose level. One female in the **150 mg/kg** dose group delivered early. A summary of other reproductive outcomes (means/litter) are provided in the table below.

Dose (mg/kg)	0	75	150	300	600
Corpora Lutea:	16.3	16.3	15.8	17.4	17.0
Implantations:	15.4	15.0	14.7	15.3	15.9
Total No. of Resorptions:	0.7	<b>0.8</b>	0.7	0.6	0.6
Total No. of Fetuses:	14.6	14.2	14.0	14.7	15.3
Total No. of Live Fetuses:	14.6	14.2	14.0	14.7	15.3
Mean Fetal Weight	5.02	5.20	5.17	5.05	4.38
Sex Ratio (No. male fetuses/No. live fetuses):	0.44	0.49	0.50	0.50	0.48

There was no evidence of either maternal or developmental toxicity at either 75 or 150 **mg/kg**.

Marginal evidence of both maternal and developmental toxicity was detected at 300 **mg/kg**. Regarding maternal toxicity, lung noise similar to that observed at 600 mg/kg was observed in 2 of 25 dams. Developmental toxicity was evident only as a slight, but not statistically significant, increase in the incidence of skeletal malformations (fused ribs and fused vertebra). No other maternal or developmental parameters were affected.

Compound-related, adverse maternal and developmental toxicity was observed at **600 mg/kg**. Maternal effects included significant reductions in maternal body weights and food consumption. Adverse clinical observations were

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significantly increased at this level as well, and included abnormal gait/staggering, lung noise (wheezing and/or rattling), irregular respiration, and lethargy. There were no remarkable postmortem findings in these dams.

Developmental toxicity was evident at this level in significantly reduced mean fetal weight, a significant increase in the incidence of fetal malformations (fused and absent ribs, fused and hemivertebra, and abnormally fused and **cleft/non-fused** sternebra), and increased fetal variations (misaligned and incompletely ossified sternebra and incompletely ossified vertebra).

A summary of gross, **soft** tissue, and skeletal anomalies are provided in the table **below**. Data are presented as number of fetuses (litters) affected.

Dose (mg/kg)	0	75	150	300	600
<b>Skeletal, Number examined</b>	366(25)	354(25)	321(23)	339(23)	351(23)
Fused ribs	0(0)	0(0)	0(0)	2(2)	9(9)
Absent ribs	0(0)	0(0)	0(0)	0(0)	3(3)
Fused vertebra	0(0)	0(0)	0(0)	2(2)	6(6)
Hemi-vertebra	0(0)	1(1)	0(0)	1(1)	8(8)
Abnormally fused sternebra	0(0)	0(0)	0(0)	0(0)	3(3)
Cleft/non-fused sternebra	1(1)	0(0)	0(0)	1(1)	6(5)
Misaligned sternebra	1(1)	1(1)	0(0)	1(1)	13(9)
Incompletely ossified sternebra	14(6)	4(2)	13(2)	12(3)	60(14)
Incompletely ossified vertebra	95(19)	119(21)	68(17)	114(22)	204(21)

Thus, the maternal and developmental no-observed-effect level (NOEL) was 1 **50 mg/kg/day**. Therefore, the results of this study indicate that glycolic acid is not likely to be uniquely toxic to the rat conceptus.

Reference:

DuPont Co. (1996). Unpublished Data, **Haskell** Laboratory Report No. 191-96 (also cited in **TSCA** fiche **OTS0572155-1**).

Munley, S. M. and M. E. Hurtt (1996). **Teratology**, **53(2):117** (Abstract No. P54).

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**Munley, S. M. et al. (1999). Drug Chem. Toxicol., 22(4):569-582.**

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

Study No. 2

**Type:**

***In vitro* Developmental Toxicity**

**Species/Strain:**

**Rat/Sprague Dawley**

**Sex/Number:**

Rat **embryo/10** per concentration (Experiment 1); 12 per concentration (Experiment 2)

Route of

Administration:

***In vitro* culture**

Exposure Period:

46 hours

Exposure Levels:

**0, 0.5, 2.5, 12.5, 25, 50 mmol/L**

Method:

Adult female time-mated Sprague Dawley rats, obtained from Charles River Breeding Laboratory, supplied the embryos used in this study. Day 10.5 conceptuses (day of sperm-positive vaginal smear or copulation plug was designated as day 0.5) were dissected **free** of decidual tissue and Reichert's membrane, leaving the visceral yolk sac and **ectoplacental** cone intact. Early **somite** stage embryos were then transferred to culture bottles (2 embryos/bottle) containing pre-warmed, pm-gassed test or control culture media. The culture bottles were maintained **in** a continuous gas flow rotating culture unit at 37°C for 46 hours. The culture medium was comprised of 75% immediately centrifuged, heat-inactivated serum collected from adult rats and 25% Dulbecco's Modified Eagle's Medium.

**In** Experiment 1, groups of 10 embryos were **cultured** in media **containing** the test substance for 46 hours. An additional group of 10 embryos was cultured with sodium valproate (**1.0 mM**) and served as positive controls. **In** Experiment 2, groups of 12 embryos were cultured for 46 hours in 1 of 4 media: control medium at **pH** 7.41, control medium titrated to **pH** 6.74 with **HCl**, medium containing 12.5 **mM glycolic acid (pH 6.74)**, or medium containing 12.5 **mM sodium glycolate (pH 7.42)**.

Upon **completion** of **the** culture period, embryos were evaluated for the presence of a beating heart and an active visceral yolk sac circulation. Embryos with a beating heart were considered viable embryos. **Morphology** was evaluated using Brown-Fabro scoring. Growth was assessed by measurement of visceral yolk sac diameter, crown-rump

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The percentage of dysmorphic embryos was significantly increased in the glycolic acid 12.5 and 25 **mM** groups. The most commonly observed morphological alteration was a striking, bilateral, **cystlike** enlargement of the maxillary process. At 12.5 **mM**, several **rostro-medial** structures of the midfacial region were observed, which included an apparent absence of olfactory rudiments, irregular protrusions extending from an area just above the expected region of the nasal process, and hypoplastic and/or widely spaced telencephalic hemispheres. Other effects observed included dysmorphogenesis of the optic vesicles and an irregular pattern of **somite** segmentation. Similar effects were observed in the surviving embryos exposed to 25 **mM** glycolic acid. In addition, a disorganized pattern of visceral yolk sac vessels was observed in embryos exposed to 25 **mM** glycolic acid. Exposure of embryos to the positive control agent resulted in abnormal **somite** segmentation, altered neural tube development (characterized by a wavy or kinked neural suture line), incomplete closure of the **otic** vesicles, disorganized vitelline (visceral yolk sac) vessels and other abnormalities.

Embryo viability was not **affected** by any of the treatments in Experiment 2. However, the percentage of embryos with active yolk sac circulation was slightly, but not significantly decreased in both the 12.5 **mM** glycolic acid (**pH** 6.74) and acidified medium (**pH** 6.74) groups. The effects observed on the growth of embryos cultured in 12.5 **mM** glycolic acid (**pH** 6.74) were virtually identical to those observed in Experiment 1, with all parameters significantly different from **pH** 7.41 controls. The same measures were also affected by culture with 12.5 **mM** sodium glycolate at **pH** 7.42, although to a slightly lesser degree. In contrast, only the protein contents of embryo and visceral yolk sac and embryo head length were significantly affected in embryos cultured in control medium titrated to **pH** 6.74. Both treatment with 12.5 **mM** glycolic acid (**pH** 6.74) and 12.5 **mM** sodium glycolate (**pH** 7.42) resulted in a significant increase in the percentage of dysmorphic embryos. The type of alterations were similar, and as seen in Experiment 1, were predominantly craniofacial. Again the maxillary process and midfacial region were the principle sites affected.

In Experiment 1, the **pH** of representative media samples (without embryos) were in the physiological range at the

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start of the culture, and remained relatively stable during the **46-hour** culture period. In Experiment 2, relatively slight changes in **pH** occurred in the 12.4 **mM** glycolic acid, 12.5 **mM** sodium glycolate, and acidic control groups. Osmolalities of 299,396, and 377 **mOsmol/kg H<sub>2</sub>O** were noted for the control, 12.5 **mM** glycolic acid, and 12.5 **mM** sodium glycolate medium, respectively. Differences between osmolality for the serum-based media used in the study versus theoretical values predicted for dilute aqueous solutions were likely due to interactions among serum components, as well as **pH** influences on the ionization of such components.

References:

Carney, E. W. et al. (1996). **Teratology**, **53:38-46**.

Carney, E. W. et al. (1995). **The Toxicologist**, **15( 1): 163** (Abstract No. 866).

Dow Chemical Company (1995). Correspondence to U. S. EPA, OTS Office, 8E Communication (April).

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.

Breslin, W. et al. (1997). **The Toxicologist**, **36 (1, Part 2):100** (Abstract 511).

Carney, E. W. et al. (1999). **Toxicol. Sci.**, **50:117-126** (cited in **NICNAS** (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Carney, E. W. et al. (1997). Unpublished Data, Dow Chemical Company, Study ID K-0025558-012 (cited in **NICNAS** (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

DuPont Co. (1995). Unpublished Data, Haskell Laboratory Report No. 96-95 (also cited in TSCA fiche **OTS0572155**).

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#### **5.4 Reproductive Toxicity**

Species/Strain: **Rats/Crl:CD®(SD)IGS BR**  
Sex/Number: Male and female/10 per sex per dose group  
Route of Administration: Cavage  
Exposure Period: 90 days of feeding plus 1 -generation reproduction  
Frequency of Treatment: Daily  
Exposure Levels: **0, 150, 300, 600 mg/kg**  
Method: Rats (**40/sex/dose** group) were administered solutions that contained 0, 150,300, or 600 **mg/kg** glycolic acid daily for 90 days. Each dosage group was divided into subchronic toxicity, immunotoxicity, neurotoxicity, and reproductive toxicity subsets (**10/sex/subset/concentration**). Details for the subchronic, immunotoxicity, and neurotoxicity subsets can be found in Section 5.2.

During the **90-day** feeding phase of the study, all rats were weighed, clinical observations were recorded, and food consumption was measured. On test day 97, animals of the reproductive toxicity subset were bred within their respective treatment groups. Each female was continually housed on a 1: 1 basis with a randomly selected male of the same dose level until copulation was observed (designated as day 0 of gestation) or until 2 weeks had elapsed. The presence of an **intravaginal** or extruded copulation plug was considered evidence of copulation. Females were allowed to deliver and rear their offspring until weaning (postpartum day 21). During the mating, gestation, and lactation phases of the study, body weights and clinical observations were recorded for male and female rats, and food consumption was recorded only for female rats during gestation. Live and dead pups in each litter were counted, and body weights and clinical observations of pups were recorded. On Day 4 postpartum, the litters were culled randomly to 8 (**4/sex** when possible). Extra pups from this culling were euthanatized and did not receive pathological evaluation. Litters of 8 pups or less were not reduced. All parental rats were sacrificed and received a gross pathological examination. The testes of each male rat were weighed. The uteri of all cohabited female rats were examined for the presence and number of implantation sites. Offspring that were found dead during the lactation period underwent gross pathological evaluation. **Weanlings** were sacrificed on postpartum day 21 and underwent gross pathological

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evaluation. Gross lesions **from** some **weanlings** were evaluated microscopically. Reproductive parameters recorded or calculated included gestation length, mating index, fecundity index, gestation index, litter survival, implantation site numbers, implantation efficiency, sex ratio, percent born alive, viability index, and lactation index.

GLP: Yes

Test Substance: Glycolic acid (tested as a 70% solution in water), purity >98%

Results: Results of the **90-day** subchronic portion of the study can be found in Section 5.2. Results of reproductive performance are detailed below.

**Significant** decreases in mean body weight of females during gestation was observed in the 300 and 600 **mg/kg** dose groups. In addition, a significant decrease in mean body weight was observed in the 600 **mg/kg** dose group on lactation day 0. Since there were no statistically significant differences in overall mean body weight gain for these females during gestation (days **0-21**), and there was an overall body weight gain during the 21-day lactation period for the 600 **mg/kg** females, it was concluded that these effects were due to preexisting body weight deficits established during the premating period. There were no differences in maternal food consumption or food efficiency during gestation; clinical observations during gestation or lactation; mating, fecundity, or gestation indices; implantation **efficiency**; or gestation length in rats dosed with any concentration of test substance. There were no test substance-related effects on litter size, pup survival, pup weight, or pup clinical signs. A summary of reproductive outcomes is provided in the table below.

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Dose (mg/kg)	0	150	300	600
Mating Index(%):	80.0	90.0	100.0	90.0
Fecundity Index (%):	75.0	88.9	90.0	66.7
Gestation Length (days):	22.3	22.6	22.4	22.7
Implantations:	13.8	13.6	13.8	13.3
Implantation efficiency (%):	84.4	97.8	88.0	87.1
Gestation Index:	100.0	100.0	100.0	100.0
Mean % Born Alive:	100.0	100.0	100.0	98.6
0-4 Day Viability %:	97.5	98.3	100.0	100.0
Lactation Index (%):	100.0	100.0	98.6	100.0
Litter Survival (%):	100.0	100.0	100.0	100.0
Sex Ratio (males):	0.58	0.52	0.41	0.48

There were no changes in organ weights or gross pathology of the reproductive system. There were compound-related gross lesions (dilation of the pelvis, calculus, chronic nephropathy, and pale discoloration) in the kidneys of male rats in the 600 mg/kg/day group that correlated microscopically with oxalate crystal nephropathy similar to that diagnosed in the subchronic toxicity animals. No compound-related gross observations were noted in the P<sub>1</sub> females or the F<sub>1</sub> weanlings.

Reference: DuPont Co. (1999). Unpublished Data, Haskell Laboratory Report No. DuPont- 1597.  
Reliability: High because a scientifically defensible or guideline method was used.

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

## 5.5 Genetic Toxicity

Type: **In vitro Bacterial Reverse Mutation Assay**  
Tester Strain: **Salmonella typhimurium TA97a, TA98, TA100, TA1535 Escherichia coli strain WP2 uvrA (pKM101)**  
Exogenous Metabolic Activation: With and without **Aroclor®-induced rat liver S9**  
Exposure Concentrations: **Salmonella typhimurium strain TA 100 and Escherichia coli**

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strain WP2 *uvrA* (pKM101): 1, 5, 10, 50, 100, 500, 1000, 2500, 5000 µg/plate

*Salmonella typhimurium* strains TA97a, TA98, TA1535: 10, 50, 100, 500, 1000, 2500, 5000 µg/plate

Method:

Concentrations of glycolic acid were evaluated in comparison to negative (solvent) controls using *Salmonella typhimurium* strain TA100 and *Escherichia coli* strain WP2 *uvrA* (pKM101). Concentrations of *Salmonella typhimurium* strains TA97a, TA98, and TA1535 were subsequently tested to complete the first trial. In a second independent assay, concentrations of glycolic acid were tested in comparison to negative (solvent) controls.

Solutions of the test substance were prepared immediately prior to treatment, and were presumed to be stable under the conditions of the study. Treatment and control solutions were not analyzed for concentration, uniformity, or stability. The stock solution used for preparation of the dosing solutions was corrected for a purity value of 70.58%.

This study consisted of 2 independent trials that assessed test substance mutagenicity. For each trial, 3 replicates were plated for each tester strain in the presence and absence of the exogenous metabolic activation system at each test substance concentration. Phosphate buffered saline (PBS) was used as the test substance solvent, diluent, and negative control. Positive controls included the following: **2-nitrofluorene (2NF)**, **N-ethyl-N-nitro-N-nitroguanidine (ENNG)**, sodium **azide (NAAZ)**, **ICR 191** acridine mutagen (**ICR 191**), **9,10-dimethyl-1,2-benzanthracene (DMBA)**, and **2-aminoanthracene (2AA)**. Treatments with the exogenous metabolic activation system were conducted by adding negative or positive control or test substance solution, metabolic activation system, and overnight culture containing approximately  $1 \times 10^8$  bacteria to top agar containing **L-histidine**, D-biotin, and **L-tryptophan**. Treatments in the absence of the metabolic activation system were the same as those in the presence of the exogenous metabolic activation system, with the exception that sterile buffer was used as a replacement for the exogenous metabolic activation system. After pouring onto the surface of minimal glucose agar plates, the top agar was allowed time to solidify, and the individually labeled plates were inverted and incubated at approximately 37°C for approximately 48 hours. When necessary, plates were

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refrigerated at  $4 \pm 3^{\circ}\text{C}$  prior to evaluation and counting of revertant colonies.

Bacterial background lawns were evaluated for evidence of test substance toxicity and precipitation. Evidence of toxicity was scored relative to the concurrent negative control plates and recorded. Revertant colonies for a given tester strain and condition were counted by an automated colony counter, unless the plate exhibited excessive toxicity. Plates with test substance precipitation that interfered with the automated colony counting were counted manually, when possible.

A test substance was classified as positive (mutagenic) if the mean number of revertants in any strain at any test substance concentration was at least 2 times greater than the mean of the concurrent vehicle control and there was a **concentration-related** increase in the mean revertants per plate in that same strain. A test substance was classified as negative (not mutagenic) if there were no test substance concentrations with a mean number of revertants that were at least 2 times greater than the mean of the concurrent vehicle control or there was no positive concentration-related increase in the mean revertants per plate in that same strain. The test article was classified equivocal if there was no consistent evidence for either a positive or negative evaluation.

GLP: Yes  
Test Substance: Glycolic acid (tested as a 70% solution in water), purity >98%.  
Results: Negative  
Remarks: **In** all assays, toxicity was observed in the bacterial background lawns, with and without **S9**, usually at 1000  $\mu\text{g}/\text{plate}$  and above. No evidence of mutagenic activity was detected in either of 2 independent trials.  
Reference: DuPont Co. (1998). Unpublished Data, Haskell Laboratory Report No. DuPont-1301.  
Reliability: High because a scientifically defensible or guideline method was used.

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

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

DuPont Co. (198 1). Unpublished Data, Haskell Laboratory Report No. **608-8 1**.

**10-Julv-2001**

Hoechst AG (1992). Unpublished Data, Report No. 92.0588 (cited in IUCLID (1998). IUCLID Data Sheet "Glycolic acid" (October 6)).

Microbiological Associates (1994). Unpublished Data, submitted by CTFA, Study No. **G94AT72.330 (95-AHA-42)**, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

Yamaguchi, T. and K. Nakagawa (1983). **Agric. Biol. Chem.** **47(11):2461-2465**.

**Type:** ***In vitro* Mouse Lymphoma Forward Mutation Assay**  
**Cell Type:** Mouse lymphoma cells (**L5 178Y**; TK locus)  
**Exogenous Metabolic Activation:** With and without **Aroclor<sup>®</sup>-induced** rat liver **S9**  
**Exposure Concentrations:** Initial assay: **39.3, 78.5, 157, 313, 625, 1250, 2500, 5000 µg/mL**  
**Confirmatory assay:** **250, 500, 1000, 2000, 2500, 3000, 4000, 5000 µg/mL**  
**Method:** The vehicle for the test substance was water in the cytotoxicity assay and was changed to Fischer's medium for the mutation assays to aid pH adjustment. Concurrent vehicle controls were performed for each portion of the assay, both with and without metabolic activation. Three vehicle control cultures were initiated in the mutation assays. The positive control substances were methyl methanesulfonate (MMS) and methylcholanthrene (MCA) for the assays without and with exogenous metabolic activation, respectively. Preparations of test substance in the vehicle were prepared fresh each day. The stock solution used for preparation of the dosing solutions was corrected for a purity value of 70.58%.  
  
Initial and **confirmatory** assays were performed without exogenous metabolic activation. In the initial assay, 8 concentrations were analyzed for mutant induction. A **confirmatory** assay was performed with concentrations clustered at the high end of the concentration range. In the presence of **S9** metabolic activation, an initial and confirmatory assay were also performed. The assay conditions consisted of vehicle controls in triplicate, 2 positive controls, and 10 different test substance dose

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levels using 1 culture per dose level. Treated cultures were eliminated during the expression period so that 8 dose levels were left for analysis of mutant induction. The appearance of the treated cultures was recorded both at the time of treatment and **after the 4-hour** treatment period. A standard expression period of 2 days was used to allow for mutant recovery, growth, and expression of the **TK<sup>+/-</sup>** phenotype. Cell densities were determined on day 1 and were adjusted to **3x10<sup>5</sup> cells/mL** in 20 mL of growth medium. If the cells in a culture failed to multiply to a density of **4x 10<sup>5</sup>** on the first **day** after treatment, the culture was not subcultured. On day 2, cell counts were again determined, and appropriate cultures were selected for cloning and mutant selection. Cultures with cell densities less than approximately **3x10<sup>5</sup> cells/mL** were deemed unacceptable due to cytotoxicity, and were not considered for selection. A total of **3x10<sup>6</sup>** cells from each selected tube was suspended in selection medium in soft agar to recover mutants. This sample was distributed into 3 dishes (100 mm each). All dishes were placed in an incubator at approximately 37°C with approximately 5% **CO<sub>2</sub>:95%** humidified air. After 12 or 13 days in the incubator, the colonies were counted. The mutant frequency was calculated as the ratio of the total number of mutant colonies found in each set of 3 mutant selection dishes to the total number of cells seeded, adjusted by the absolute selection cloning efficiency.

The test substance was evaluated as positive, negative, or equivocal in this assay. The test substance was classified positive (induced gene mutations) if dose-dependent increases of **2-fold** or greater in mutant frequency were obtained over the concurrent background mutant frequency (average mutant frequency of the vehicle control cultures). It was desirable to obtain this relationship for at least 3 doses, but this goal depended on the dose steps chosen for the assay and toxicity at which mutagenic activity appeared. The dose dependent requirement was waived if a large increase in mutant frequency (**4-fold** or higher) was obtained for a single dose at or near the highest testable toxicity. However, for the test substance to be evaluated as positive, any increases must have been repeated in the **2<sup>nd</sup>** trial.

The test substance was classified negative in a single trial if a **2-fold** increase in mutant frequency was not observed for (1) a range of doses that extended to toxicities causing **10-20%** relative total growth, (2) for relative nontoxic test

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substances, a range of doses that extended to the maximum concentration of 5 **mg/mL**, (3) a range of doses that extended to a level approximately twice the solubility limit in **culture** medium, or (4) the increase(s) were not repeatable in a confirmatory trial. The test article was classified equivocal if there was no consistent evidence for either a positive or negative evaluation.

GLP: **Yes**  
Test Substance: **\*Glycolic** acid (tested as a 70% **solution** in water), purity **>98%**.  
Results: **Equivocal:**

Without **S9** metabolic activation: **Negative**.

With **S9** metabolic activation: **Negative** at concentrations of 26 **mM** and below. **Positive** with metabolic activation at concentrations of 33 **mM** and above.

Remarks: See remarks for additional information.  
The test substance did not induce forward mutations at **the TK** locus in **L5178Y** mouse lymphoma cells under the nonactivation conditions. The test substance produced increases in mutant frequency in the presence of **S9** metabolic activation at high concentrations only (2500-5000 **µg/mL**), which corresponds to 33-66 **mM** concentrations. However, no increases in mutant frequency were observed at or below 2000 **µg/mL** (26 **mM**). The recommended maximum concentrations specified in the OECD testing guideline is 10 **mM** for this assay. Therefore, the positive response was only observed at excessively high concentrations. The average cloning efficiencies for the vehicle controls varied from 93.9% and 77.7% without activation to **95.0%** to 77.2% with activation. The positive control cultures, MMS (nonactivation) and MCA (activation) induced large increases in mutant frequency that were greatly in excess of the minimum criteria.

Reference: DuPont Co. (1998). Unpublished Data, Haskell Laboratory **Report No.** DuPont-1616.

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

#### **Additional Reference for In *vitro* Genetic Toxicity Assays:**

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.

**10-Julv-2001**

Microbiological Associates (1994). Unpublished Data, submitted by CTFA, Study No. **G94AT72501 (95-AHA-41)**, Washington, DC, Cosmetic Ingredient Review (cited in NICNAS (2000). Glycolic Acid: Priority Existing Chemical Assessment Report No. 12, National Industrial Chemicals Notification and Assessment Scheme, Commonwealth of Australia).

**Type:** *In vivo* **Mouse** Micronucleus Assay  
**Species/Strain:** **Mice/Crl:CD-1<sup>®</sup>(ICR)BR**  
**Sex/Number:** Male and **female/5** per sex for the low and mid doses; 10 per sex for the control; 15 males and 13 females for the high dose

Route of Administration: **Oral** intubation  
Exposure Concentrations: **300, 600, 1200 mg/kg** (low, mid, and high doses, respectively) for males  
**400, 800, 1600 mg/kg** (low, mid, and high doses, respectively) for females

**Method:** Immediately prior to dosing, glycolic acid solutions, adjusted for purity, were prepared in water. The stock solution used for preparation of the dosing solutions was corrected for a purity value of 70.58%. The corresponding vehicle control was water, and the positive control was cyclophosphamide (CP). All mice were dosed by intragastric intubation. The number of mice in the high dose groups exceeded 10 mice/sex to ensure a minimal number survived for evaluation of micronuclei in the event of mortality. Body weights and clinical signs were recorded. Bone marrow smears were prepared from 5 mice/sex from the control and all treatment groups 24 hours post-dosing, and from the control and high-dose treatment groups 48 hours post-dosing. Bone marrow smears were examined using incident light fluorescence microscopy. Color was used to distinguish **PCEs** (polychromatic erythrocytes) from **NCEs** (normochromatic erythrocytes). **PCEs** (2000 per animal) were evaluated for the presence of micronuclei. Cellular inclusions that were irregularly shaped or stained, or out of the focal plane of the cell, were considered artifacts and were not included in the micronuclei counts. The unit of scoring was the micronucleated cell; therefore, **PCEs** with more than 1 micronucleus were scored as a single MNPCE. Micronucleated **NCEs** were counted in each optic **field** while **scoring** the 2000 **PCEs**. Additionally, **the** number of **PCEs** among 1000 erythrocytes was recorded for each animal.

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For the test substance to be judged negative, no statistically significant increases in MNPCEs above the concurrent vehicle control values could occur at any dose level or sampling time. In order for a test substance to be judged positive, statistically significant increases in **MNPCEs** above concurrent vehicle control values must have been observed at more than one sampling time, or in both sexes. However, the final analysis was based on scientific judgement and results not meeting the indicated criteria for positive or negative **findings** were evaluated on a case-by-case basis.

GLP: Yes  
Test Substance: Glycolic acid (tested as a 70% solution in water), purity >98%.  
Results: Negative  
Remarks: A total of 5 males and 3 females **from** the highest dose groups (1200 and **1600 mg/kg**, respectively) were found dead 1 or 2 days post-dosing. There were no statistically significant decreases in body weight gain in either male or female mice administered **glycolic** acid. Within approximately 2 hours post-dosing, clinical signs of systemic toxicity, including lethargy, moribundity, and/or abnormal gait were observed in a few mice **from** the groups that received the highest doses of glycolic acid (**5/15** males in the 1200 **mg/kg** group, and **2/13** females in the 1600 **mg/kg** group). Lethargy and/or moribundity continued to be observed at a low frequency in these groups up to 2 days post-dosing. There were no clinical signs of toxicity or statistically significant body weight gain decrements in CP-treated mice of either sex.

No statistically significant increases in micronucleated PCE frequency were observed in any test substance-treated group at either time point. The proportions of **PCEs** among 1000 erythrocytes were decreased approximately 18 and 25% below concurrent control values in the high dose male and female mice, respectively, at the **48-hour** time point. Although not statistically significant, the depressions in this parameter might be indicative of bone marrow toxicity. Glycolic acid did not induce a statistically significant increase in micronucleated **PCEs** in mouse bone marrow. As expected, there were statistically significant increases in **MNPCE** frequency in male and female mice treated with CP. No statistically significant depressions in the **PCE/NCE** ratio were found in either CP-treated male or female mice.

Reference: DuPont Co. (1998). Unpublished Data, Haskell Laboratory

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Report No. DuPont- 1197.  
Reliability: High because a scientifically defensible **or** guideline method  
was used.

**Additional References for *In vivo* Mouse Micronucleus Assay:** None Found.