

**F. PHYSICAL, CHEMICAL DESCRIPTION**

AR201-13131B

**1. MELTING POINT**

Test substance: 2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3

**NOT APPLICABLE**

**F. PHYSICAL CHEMICAL DESCRIPTION****1. MELTING POINT**

Test substance: 2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3

**NOT APPLICABLE**

## 2. BOILING POINT

Test substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3
Method:	Estimated by the MPBPWIN Program (v.1.40) <sup>1</sup> , using the adapted Stein and Brown Method.
GLP:	Not applicable to estimations
Year:	2001
Results:	186.88 °C (with decomposition)
Remarks:	The boiling point calculation by an accepted method is assigned a reliability code of 2f according to the criteria established by Klimisch <i>et al.</i> (1997) <sup>2</sup> .
References:	<sup>1</sup> Syracuse Research Corporation, Syracuse, NY  Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.  <sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. <i>Regulatory Toxicology and Pharmacology</i> . 25: 1-5, 1997. See Listing of Codes, p.34.

### 3. VAPOR PRESSURE

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3
Method:	Estimated by the MPBPWIN Program (v.1.40) <sup>1</sup> , using mean of Antoine and Grain Methods.
GLP:	Not applicable to estimations
Year:	2001
Results:	0.6 mmHg @ 25 °C
Remark:	The vapor pressure calculated by an accepted method is assigned a reliability code of 2f according to the criteria established by Klimisch <i>et al.</i> (1997) <sup>2</sup> .

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Test Substance:	60% 2-amino-2,3-dimethylbutanenitrile in Toluene
Method:	The vapor pressure was measured using a static method. The sample was placed in a glass cell and degassed using five freeze-pump-thaw cycles. The sample temperature was measured to $\pm 0.01$ degrees C with a Hewlett-Packard Quartz Thermometer and controlled to $\pm 1$ degree C with a Blue-M forced air oven. The pressure was measured with a MKS Baratron capacitance transducer. The sample was stable during the experiment with no discoloration and it gave stable pressure reading once thermal equilibration was achieved.
GLP:	No
Year:	1988
Results:	23.42 mmHg @ 25 °C <sup>3</sup>
Remark:	This study is assigned a reliability code of 2e according to the criteria established by Klimisch <i>et al.</i> (1997) <sup>2</sup> . It was not conducted under GLP or OECD guidelines but generally meets scientific standards, is well documented and is accepted for assessment.

## References:

<sup>1</sup>Syracuse Research Corporation, Syracuse, NY

Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998

<sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25: 1-5, 1997.

See Listing of Codes, p.34.

<sup>3</sup>American Cyanamid Company, Stamford Research Center, 1988

**4. PARTITION COEFFICIENT**

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3
Method:	Estimated by the KowWin Program (v.1.66) <sup>1</sup>
GLP:	Not applicable to estimations
Year:	2001
Results:	Log Kow = 0.87
Remark:	The partition coefficient calculated by an accepted method is assigned a reliability code of 2f according to the criteria established by Klimisch et al. (1997) <sup>2</sup> .
References:	<sup>1</sup> Syracuse Research Corporation, Syracuse, NY  Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998  <sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. <i>Regulatory Toxicology and Pharmacology</i> . 25: 1-5, 1997. See Listing of Codes, p.34.

**5. WATER SOLUBILITY**

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3
Method:	Estimated from Kow with WSKOW (v1.40) <sup>1</sup> : KowWin Estimate
GLP:	Not applicable to estimations
Year:	2001
Results:	1.07 E+5 mg/L @ 25°C
Remark:	The water solubility calculated by an accepted method is assigned a reliability code of 2f according to the criteria established by Klimisch et al. (1997). <sup>2</sup>
References:	<sup>1</sup> Syracuse Research Corporation, Syracuse, NY  Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998  <sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. <i>Regulatory Toxicology and Pharmacology</i> . 25: 1-5, 1997. See Listing of Codes, p.34.

**G. ENVIRONMENTAL FATE DATA****1. PHOTODEGRADATION**

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3
Method:	Estimated by the AOP program (v1.90) <sup>1</sup> , which estimates rate constants and half-lives of atmospheric reactions of organic compounds with hydroxyl radicals and ozone in the atmosphere.
GLP:	Not applicable to estimations
Year:	2001
Results:	For reaction with hydroxyl radicals, the predicted half-life of the chemical is relatively rapid.  Rate constant: $2.888 \times 10^{-12}$ cm <sup>3</sup> /molecule-sec Half-life: 44.443 hours
Remark:	The photodegradation rate calculated by an accepted method is assigned a reliability code of 2f according to the criteria established by Klimisch et al. (1997). <sup>2</sup>
References:	<sup>1</sup> Syracuse Research Corporation, Syracuse, NY  Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998  <sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. <i>Regulatory Toxicology and Pharmacology</i> . 25: 1-5, 1997. See Listing of Codes, p.34.

## 2. HYDROLYSIS

Test Substance: 2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3

Method: Estimated by the HYDROWIN program (v1.67)<sup>1</sup>.

GLP: Not applicable to estimations

Year: 2001

Results: No estimate available.

Remark: This program was not able to estimate a hydrolysis rate constant for this type of chemical structure. However, as manufactured, 2-amino-2,3-dimethylbutanenitrile is prepared as an 80% solution in toluene and this solution will partially hydrolyze in water by producing CN<sup>-</sup>, which will be detectable immediately. A small fraction of the 2-amino-2,3-dimethylbutanenitrile dissociates under ambient conditions, whether as neat (100%) liquid or in solution with non-reactive organic solvents such as toluene. CN<sup>-</sup> is a product of the dissociation of 2-amino-2,3-dimethylbutanenitrile and will be present in a low concentration in equilibrium with 2-amino-2,3-dimethylbutanenitrile under all expected conditions.

Aqueous wastes containing 2-amino-2,3-dimethylbutanenitrile, when commingled with a wastestream that is maintained at a pH of at least 10 by the addition of caustic, chemically decomposes the 2-amino-2,3-dimethylbutanenitrile to CN<sup>-</sup>, ammonia, and methyl isopropyl ketone. Thus indicating that with pH increase the material decomposes.

Reference: <sup>1</sup>Syracuse Research Corporation, Syracuse, NY

Pollution Prevention (P2) Assessment Framework, U.S.  
Environmental Protection Agency, Office of Pollution Prevention  
and Toxics (Draft), 1998

### 3. TRANSPORT (FUGACITY)

Test Substance: 2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3

Method: Estimated by the Level III Fugacity Model (Full-Output)

GLP: Not applicable to estimations

Year: 2001

Results: Distribution using Level III Fugacity Model:

Air:	0.158%
Water:	46.2%
Soil:	53.5%
Sediment:	0.0913%

Remark: The fugacity calculated by an accepted method is assigned a reliability code of 2f according to the criteria established by Klimisch et al. (1997).<sup>2</sup>

References: <sup>1</sup>Syracuse Research Corporation, Syracuse, NY

Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998

<sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25: 1-5, 1997.  
See Listing of Codes, p.34.

#### 4. BIODEGRADATION

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3
Method:	Estimated by STP Fugacity Model: Predicted Fate in a Wastewater Treatment Facility.
GLP:	Not applicable to estimations
Year:	2001
Results:	Total biodegradation is predicted to be 0.09 %. The material is not considered readily biodegradable.
Remark:	The biodegradation rate calculated by an accepted method is assigned a reliability code of 2f according to the criteria established by Klimisch et al. (1997). <sup>2</sup>
References:	<sup>1</sup> Syracuse Research Corporation, Syracuse, NY  Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998  <sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. <i>Regulatory Toxicology and Pharmacology</i> . 25: 1-5, 1997. See Listing of Codes, p.34.

## H. ECOTOXICITY DATA

### 1. ACUTE TOXICITY TO FISH<sup>1</sup>

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3
Method:	Patterned after EPA-660-3-75-009. ABC Laboratories Protocol 7601 (American Cyanamid Protocol 981-83-140).
Type:	static
Species:	Lepomis macrochirus (Fish, fresh water)
Exposure period:	96 hour(s)
Analytical monitoring:	Exposures based on nominal concentrations
Year:	1984
GLP:	The study was conducted following the intent of Good Laboratory Practices.
Results:	NOEC = 0.56 mg/L LC50 = 0.75 mg/L
Remark:	This study is assigned a reliability code of 2c according to the criteria established by Klimisch <i>et al.</i> (1997) <sup>2</sup> .

#### Summary details:

The static fish bioassay was conducted in five gallon glass vessels containing 15 liters of soft reconstituted water. 10 fish with a mean weight of 0.34 g and a mean length of 25 mm were used for each test concentration. The test vessels were kept in a water bath at 22 ( $\pm$ 1) C. A 48-hour range-finding test was conducted to determine the concentration range for the definitive study. The preliminary test concentrations were set at 0.1, 1.0, and 10 mg/L. Based on the results of the preliminary testing, five test concentrations were selected, 0.10, 0.18, 0.32, 0.56, and 1.0 mg/L. Test concentrations were prepared by preparing a stock solution in deionized water and serially diluting to obtain desired concentration. All results were based on the nominal concentrations. The bluegill sunfish (*Lepomis macrochirus*) were challenged with a reference compound, Antimycin A, to verify that the fish were in good condition. The 96-hour LC50 for bluegill sunfish exposed to the control substance was  $1.2 \times 10^{-4}$  mg/L, which indicates that the fish were in good condition. The fish were observed once every 24 hours for mortality and abnormal effects. The no-effect concentration for the test material, based on the lack of mortality and abnormal effects, was estimated to be 0.56 mg/L after 96 hours. All fish in the 1.0 mg/L test concentration died on or before the 24-hour observation period. Water quality parameters of temperature, dissolved oxygen and pH were measured throughout the test and

were within acceptable limits. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized LC50 program developed by Stephan, 1978<sup>3</sup>. This program calculated the LC50 statistic and its 95% C.L. using the binomial and the moving average tests, respectively. The method of calculation selected for use was that which gave the narrowest confidence limits for the LC50.

- References:
- <sup>1</sup>ABC Laboratories Report # 31250 to American Cyanamid Company, 1984.
- <sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25: 1-5, 1997. See Listing of Codes, p.34.
- <sup>3</sup>Stephan, C.E., Busch, K., Smith, R., Burke, J. and Andrew, R. A computer program for calculating an LC50. U.S. E.P.A., Duluth, Minnesota, pre-publication manuscript, August, 1978.

## 2. ACUTE TOXICITY TO AQUATIC INVERTEBRATES<sup>1</sup>

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3
Method:	Based on methods outlined in the Committee on Methods for Toxicity Test with Aquatic Organisms, USEPA 660/3-75009. ABC Laboratories Protocol 7806 (American Cyanamid Protocol 981-83-137).
Type:	static
Species:	Daphnia magna (Crustacea)
Exposure period:	48 hour(s)
Analytical monitoring:	Exposures based on nominal concentrations
Year:	1984
GLP:	The study was conducted following the intent of the Good Laboratory Practice Regulations.
Results:	NOEC = 3.2 mg/L EC50 = 6.9 mg/L
Remark:	This study is assigned a reliability code of 2c according to the criteria established by Klimisch et al. (1997) <sup>2</sup> .

### Summary details:

The static *Daphnia magna* bioassay was conducted in 250 ml glass beakers, 10 daphnids/beaker, containing 200 ml of ABC well water. These vessels were kept at 20 ( $\pm$  2) C. The lighting was maintained at 50-70 foot-candles on a 16 hour daylight photoperiod. An initial range-finding test was conducted to determine the concentration range for the definitive study. The preliminary test concentrations were set at 0.1, 1.0, and 10 mg/L. Based on the results of the preliminary testing, five test concentrations were selected and tested in duplicate, 0 (control), 0.56, 1.0, 1.8, 3.2, 5.6, and 10 mg/L. Test concentrations were prepared by preparing a stock solution in deionized water and serially diluting to obtain desired concentrations. All results were based on the nominal concentrations. Water quality parameters of temperature, dissolved oxygen and pH were measured at the termination of the test and were within acceptable limits. The dissolved oxygen concentrations, which ranged between 8.4 and 8.8 mg/l, were considered adequate for testing. The pH values of the treated chambers were consistent with the control and ranged from 8.2 to 8.7. The no-effect concentration based on the lack of mortality and abnormal effects was 3.2 mg/l. The abnormal effects of mortality and/or daphnids lying on the bottom were observed after 24 and 48 hours of exposure in the 5.6 mg/l (24 hr-2/20 dead and 48-hr-3/20 dead) and 10 mg/l (24 hr-15/20 and 48-hr-20/20 dead) test concentrations. Statistical analysis of

the concentration vs. effect data was obtained by employing a computerized LC50 program developed by Stephan, 1978<sup>3</sup>. This program calculated the LC50 statistic and its 95% C.L. using the binomial and the moving average tests. The method of calculation selected for use was that which gave the narrowest confidence limits for the LC50.

- References:
- <sup>1</sup>ABC Laboratories Report # 31251 to American Cyanamid Company, 1984.
- <sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25: 1-5, 1997.  
See Listing of Codes, p.34.
- <sup>3</sup>Stephan, C.E., Busch, K., Smith, R., Burke, J. and Andrew, R. A computer program for calculating an LC50. U.S. E.P.A., Duluth, Minnesota, pre-publication manuscript, August, 1978.

### 3. TOXICITY TO AQUATIC PLANTS<sup>1</sup>

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3
Method:	Patterned after EPA 600/9-78-016/OTS/ASTM. ABC Laboratories Protocol 8004.
Species:	<i>Selenastrum capricornutum</i> (Algae)
Endpoint:	growth rate
Exposure period:	96 hour(s)
Analytical monitoring:	Exposures based on nominal concentrations
Year:	1984
GLP:	The study was conducted following the intent of Good Laboratory Practices
Results:	NOEC = 0.10 mg/L EC50 = 0.36 mg/L (C.L. = 0.24- 0.52 mg/L)
Remark:	This study is assigned a reliability code of 2c according to the criteria established by Klimisch et al. (1997) <sup>2</sup> .

#### Summary details:

Temperature and light readings were measured throughout the test and were within acceptable limits. The static algal toxicity study on *Selenastrum capricornutum* was conducted in 250 mL Erlenmeyer flasks containing 100 mL of synthetic algal nutrient medium. This media was composed of 1.0 mL of a salt solution diluted to a final volume of 1,000 mL of deionized water. The deionized water was filtered through a millipore Milli-Q water purification system. After the media was prepared, the pH was adjusted to 7.5 and filter-sterilized through a 0.45 um filter. To each flask was added 1 mL of algal inoculum containing  $2 \times 10^6 \pm 10\%$  cells. The test vessels were incubated for 96 hours at  $24 \pm 2^\circ\text{C}$  under continuous "cool white" fluorescent light and constant shaking. Temperature and light intensity were monitored throughout the study. Log phase growth was confirmed at 96-hours with a count of  $6.9 \times 10^5$  cells/ml in the control. A 96-hour range finding study was conducted to determine the concentration range for the definitive study. Based on the results of the range-finder, test concentrations were set at 0, 0.01, 0.1, 0.5, 1.0, and 10 mg/L. Test flasks were prepared in triplicate for each test concentrations and the control. Test concentrations were prepared by preparing a stock solution in deionized water and serially diluting to obtain desired concentration. Gravimetric determinations of algal growth at each test concentration (0, 0.01, 0.1, 0.5, 1.0, and 10 mg/L) indicated percent effected as 7, 7, 7, 58, 95, and 100, respective to the concentrations tested. The no-effect level for the test

compound was 0.10 mg/l. The EC50 was 0.36 mg/L. Statistical analysis of the concentration vs. effect data was obtained by employing a computerized LC50 program developed by Stephan, 1978<sup>3</sup>. This program calculated the LC50 statistic and its 95% C.L. using the moving average test. The method of calculation selected for use was that which gave the narrowest confidence limits for the LC50. The no effect level was determined by using ANOVA and a multiple means comparison test (Fisher's LSD).

## References:

<sup>1</sup>ABC Laboratories Report # 31252 to American Cyanamid Company, 1984.

<sup>2</sup>Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25: 1-5, 1997.  
See Listing of Codes, p.34.

<sup>3</sup>Stephan, C.E., Busch, K., Smith, R., Burke, J. and Andrew, R. A computer program for calculating an LC50. U.S. E.P.A., Duluth, Minnesota, pre-publication manuscript, August, 1978.

**I. MAMMALIAN TOXICITY****1. ACUTE ORAL TOXICITY<sup>1</sup>**

Test Substance: 2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3

Method: Animals housed at room temperature, 5/cage, fasted 18 hours before dosing. Test material suspended in corn oil. Animals dosed by oral gavage and observed several times after dosing and twice daily over a 14-day period for physical condition and mortality.

Type: oral LD50

Species/Strain: rat/ Sprague-Dawley

Sex: male

Number of animals: 10

Vehicle: corn oil

Year: 1989

GLP: no

Results: LD50 = 83 mg/kg bw

Remark: This study is assigned a reliability code of 2e according to the criteria established by Klimisch et al. (1997). It was not conducted under GLP or OECD guidelines but generally meets scientific standards, is well documented and is accepted for assessment.

**Summary details:**

Ten male rats received neat 2-amino-2,3-dimethylbutanenitrile by gavage in corn oil (5% w/v) at concentrations of 31.3, 62.5, and 125 mg/kg. Toxic signs seen in all ten animals at the highest dose and in one animal at the intermediate dose included tremors, tonic convulsions, salivation, and prostration. All animals in the 125 mg/kg dose group and 1 of the rats in the 62.5 mg/kg dose group died within 8 hours of dosing.

References: <sup>1</sup>Acute Oral Toxicity of CL 94,149. American Cyanamid Company, March 4, 1983.

<sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25: 1-5, 1997. See Listing of Codes, p.34.

**2. ACUTE INHALATION TOXICITY**4-HOUR EXPOSURE<sup>1</sup>

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3 (96% 2-amino-2,3-dimethylbutanenitrile in toluene)
Method:	OECD Guideline 403 "Acute Inhalation Toxicity"
Type:	inhalation LC50
Species/Strain:	rat/Sprague-Dawley
Sex:	male/female
Number of animals:	40
Exposure time:	4 hour(s)
Year:	1988
GLP:	yes
Results:	4 hour inhalation LC50 = 73 (67 – 79) ppm
Remark:	This study is assigned a reliability code of 1a according to the criteria established by Klimisch et al. (1997) <sup>2</sup> . It was conducted under OECD guidelines.

## Summary details:

Each group, containing five male and five female rats, was exposed once for 4 hours to vapor dynamically generated from 2-amino-2,3-dimethylbutanenitrile. The chamber atmosphere was monitored for 2-amino-2,3-dimethylbutanenitrile and hydrogen cyanide. The mean concentrations of 2-amino-2,3-dimethylbutanenitrile and (HCN) for the four 4-hour exposures were 77 (6), 71 (8), 58 (4) and 21 (<2) ppm. Mortality was observed in the 71 (40%) and 77 (70%) ppm groups. All deaths occurred on the day of exposure. Clinical signs were observed on the day of exposure for all groups except the 21 ppm group and included hypoactivity, ataxia, prostration, and signs of respiratory irritation. Hypoactivity during exposure was the only clinical sign seen in rats in the 58 ppm group. Animals were observed for the 14-day postexposure period and had no clinical signs of toxicity. Body weight gains were observed for all survivors on days 7 and 14. No macroscopic lesions were observed in the remaining rats that died or in the rats killed at the end of the 2-week recovery period.

*1-HOUR EXPOSURE<sup>1</sup>*

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3 (96% 2-amino-2,3-dimethylbutanenitrile in toluene)
Method:	OECD Guideline 403 "Acute Inhalation Toxicity"
Type:	LC50
Species/Strain:	rat/ Sprague-Dawley
Sex:	male/female
Number of animals:	30
Exposure time:	1 hour(s)
Year:	1988
GLP:	yes
Results:	1-hour inhalation LC50 = 92 (87 – 97) ppm
Remark:	This study is assigned a reliability code of 1a according to the criteria established by Klimisch et al. (1997) <sup>2</sup> . It was conducted under OECD guidelines.

## Summary details:

Each group, containing five male and five female rats, was exposed once for 1 hour to vapor dynamically generated from 2-amino-2,3-dimethylbutanenitrile. The chamber atmosphere was monitored for 2-amino-2,3-dimethylbutanenitrile and hydrogen cyanide. The mean concentrations of 2-amino-2,3-dimethylbutanenitrile and (HCN) for the three 1-hour exposures were 109 (12), 75 (4), and 63 (3) ppm. Mortality was observed in the 109 ppm group (9/10 rats died). All deaths occurred on the day of exposure. Clinical signs were observed on the day of exposure for all groups except the 63 ppm group and included hypoactivity, ataxia, prostration, and signs of respiratory irritation. Animals were observed for the 14-day postexposure period and had no clinical signs of toxicity. Body weight gains were observed for all survivors on days 7 and 14. No macroscopic lesions were observed in the remaining rats that died or in the rats killed at the end of the 2-week recovery period.

References: <sup>1</sup>Bushy Run Research Center Report # 51-611 for American Cyanamid Company, 1988.

<sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25: 1-5, 1997.  
See Listing of Codes, p.34.

### 3. ACUTE DERMAL TOXICITY<sup>1</sup>

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3
Method:	Rabbits individually quarantined 3 days prior to test. Animals fed <i>ad libitum</i> during quarantine and study. One day prior to test, the animals are shaved. Test substance applied to clipped site and held in place by a plastic wrap and napped filter cloth. Test site is wiped clean after 24 hour exposure period. Animals are observed for physical condition and mortality on the day of test material application and twice daily for 14 days.
Type:	LD50
Species/Strain:	rabbit/New Zealand white
Sex:	male
Number of animals:	25
Year:	1989
GLP:	no
Results:	dermal LD50 = 23 mg/kg bw (16-32 mg/kg)
Remark:	This study is assigned a reliability code of 2e according to the criteria established by Klimisch <i>et al.</i> (1997) <sup>2</sup> . It was not conducted under GLP or OECD guidelines but generally meets scientific standards, is well documented and is accepted for assessment.

#### Summary details:

Neat 2-amino-2,3-dimethylbutanenitrile was applied at doses of 12.5, 25, 50, 100, and 200 mg/kg to the shaved skin of 5 groups of five male albino rabbits then covered with an occlusive wrap for 24 hours. Animals were observed for 14 days. All deaths occurred within 24 hours of dose application. All of the animals in the 200, 100, and 50 mg/kg dose groups died. 3/5 rabbits in the 25 mg/kg group died. Gross autopsy not performed. Signs of toxicity observed in all animals at all dose levels included ataxia and prostration.

References: <sup>1</sup>Acute Dermal Toxicity of CL 94,149. American Cyanamid Company, March 4, 1983.

<sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25: 1-5, 1997. See Listing of Codes, p.34.

**4. REPEATED DOSE TOXICITY<sup>1</sup>**

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3
Method:	28-day Dermal Toxicity
Species/Strain:	rat/Charles-River CD (Sprague-Dawley derived)
Sex:	male/female
Route of administration:	dermal
Exposure period:	4 weeks
Frequency of treatment:	5 days/week, 6 hrs/day
Post obs. period:	2 times/day for morbidity and mortality
Doses:	0, 3, 10, and 30 mg/kg
Control group:	yes
Year:	1984
GLP:	yes
Results:	NOAEL = 3 mg/kg bw LOAEL = 10 mg/kg bw
Remark:	This study is assigned a reliability code of 1b according to the criteria established by Klimisch <i>et al.</i> (1997) <sup>2</sup> . It was not conducted under OECD guidelines but was conducted under GLP.

**Summary details:**

A 28-day repeated dermal neurotoxicity study with 94.2% 2-amino-2,3-dimethylbutanenitrile was conducted to assess its potential to cause systemic toxicity and adverse effects on the nervous system. 2-amino-2,3-dimethylbutanenitrile was administered dermally to rats (5/sex/group), at concentrations of 0, 3, 10, and 30 mg/kg (0, 3.578, 11.932, and 35.775 µl/kg) for 28 days (5 days/week, 6 hrs/day for 4 weeks). Test material was applied by gentle inunction over the clipped area of unabraded skin. Dosages adjusted at 3-day intervals to accommodate body weight changes. The treated area was covered w/ an impervious patch. After 6 hrs, the patch was removed and the treated area thoroughly cleansed. Detailed observations, body weights, and food consumption values were recorded at 3-day intervals. All rats survived the experimental period. There were no overt signs of toxicity observed at any treatment level; body weight gain, diet consumption, hematology and clinical chemistry values were comparable

across all groups. All animals were perfused with 10% buffered neutral formalin solution prior to necropsy. The weights of the liver, kidney, heart, thyroid glands, brain, and gonads were recorded. A statistically significant increase in absolute thyroid weights was observed in male rats at all treatment levels. Thyroid weights for females were somewhat increased though not significantly. Relative thyroid weights were also somewhat increased at all levels in both sexes with a significant increase in males at the 3 mg/kg level. Subsequent histopathology failed to find any pathologic change that would account for this finding. No other significant organ weight changes were observed at any treatment level. No test article related gross or microscopic lesions were observed in the tissues samples from the adrenal gland, bone marrow, brain, eye and optic nerve, heart, liver, kidneys, lung, ovary, skeletal muscle, sciatic nerve, skin, spinal cord, testes, thyroid glands, and uterus. Skin irritation, consisting of mild erythema, eschar formation, dry and/or flaky skin, and small sores were seen at the application site of rats in the 10 and 30 mg/kg dose groups. There were no overt signs of neurotoxicity (no lacrimation, no salivation, no tremors, no convulsions, no increased urination, no diarrhea, no piloerection) at any treatment level. Skin irritation, consisting of erythema, eschar formation, dry and/or flaky skin and small sores were observed at the application site in the 10 and 30 mg/kg groups. No significant irritation was seen in rats in the 3 mg/kg dose group. The authors of this study therefore concluded that the NOEL is 3 mg/kg. As the intent of the repeated exposure dermal study is to assess systemic toxicity following dermal application of the test material, and as no evidence of systemic toxicity was seen at the high dose, one could conclude that 30 mg/kg did not produce systemic toxicity or neurotoxicity and should be considered a dermal NOEL.

References:

<sup>1</sup>AC 94,149: A 28-day Dermal Rat Neurotoxicity Study, American Cyanamid Company, Report AX84-1, 1984

<sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25: 1-5, 1997.

See Listing of Codes, p.34.

## 5. DEVELOPMENTAL TOXICITY

Test Substance: 2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3

The purpose of **OECD Guideline 414**, Teratogenicity, is to assess the potential hazard to the unborn which may arise from exposure of the mother during pregnancy. Due to its high degree of acute toxicity and to the unlikelihood of this exposure scenario, we believe that an animal study of this nature would not change the already strict safeguards in place for the safe manufacture, handling, and transport of this material. Based on the surrogate data below, additional toxicity testing on 2-amino-2,3-dimethylbutanenitrile is not warranted.

2-amino-2,3-dimethylbutanenitrile is an aliphatic nitrile with one amino and two methyl side groups. In a hierarchy of aliphatic nitriles, aminonitrile would fall into the sub-family of saturated nitriles. The developmental toxicity potential of several aliphatic (saturated and unsaturated) nitriles has been investigated in both *in vitro* and *in vivo* studies (Saillenfait 2000<sup>1</sup>, Saillenfait 1993<sup>2</sup>, Willhite 1981<sup>3</sup>). Although route and duration of exposure can affect the degree of toxicity, each nitrile was demonstrated to induce adverse effects on the offspring either in the whole animal model or in *in vitro* studies. Mechanistic studies were not performed, however, the liberation of cyanide via biotransformation has been implicated as a possible mechanism of the developmental toxic effects produced by some nitriles after maternal acute exposure. In studies with acrylonitrile or propionitrile, maternal administration of thiosulfate, a cyanide antagonist, provided partial protection against the teratogenic effects of these materials (Willhite 1981). This suggests that maternal production of cyanide may contribute to the developmental toxicity of nitriles.

Based on the data available, it can reasonably be assumed that all nitriles would have the potential to produce similar adverse effects of embryoletality, fetotoxicity and teratogenicity in laboratory animals.

Common Name	Chemical Structure
<b>Saturated</b>	
Acetonitrile	CH <sub>3</sub> -CN
Propionitrile	CH <sub>3</sub> -CH <sub>2</sub> -CN
Isobutyronitrile	CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CN
n-Butyronitrile	CH <sub>3</sub> -CH(CH <sub>2</sub> )-CN
<b>2-amino-2,3-dimethylbutanenitrile</b>	CH <sub>3</sub> -C(CH <sub>3</sub> )(NH <sub>2</sub> )-CH(CH <sub>3</sub> )-CN
<b>Unsaturated</b>	
Acrylonitrile	CH <sub>2</sub> =CH-CN
Methacrylonitrile	CH <sub>2</sub> =C(CH <sub>3</sub> )-CN
Allylnitrile	CH <sub>2</sub> =CH-CH <sub>2</sub> -CN
<i>cis</i> -2-Pentenenitrile	CH <sub>3</sub> -CH <sub>2</sub> -CH=CH-CN
2-Chloroacrylonitrile	CH <sub>2</sub> =C(Cl)-CN

**Abstract Summaries:**

Following intraperitoneal injection of maternally toxic doses of either acrylonitrile or propionitrile on Day 8 of gestation in the hamster exencephaly, encephalocoels, and rib fusions and bifurcations were induced in the offspring. When animals were co-treated with sodium thiosulfate, dams and offspring were protected against toxicity except in the highest exposure groups, in which the nitrile may have overwhelmed the protective capacity of the hepatic rhodanese system to utilize sodium thiosulfate in converting liberated HCN to thiocyanide. This observation suggests that the teratogenic effects of both nitriles evaluated are related to the metabolic release of cyanide (Willhite 1981).

The relative developmental toxicities of eight inhaled aliphatic nitriles were investigated in rats. The nitriles studied included acetonitrile, propionitrile, n-butyronitrile, isobutyronitrile, acrylonitrile, methacrylonitrile, allylnitrile, and 2-chloroacrylonitrile. Exposures were 6 hours/day during Days 6 to 20 of gestation. Results indicated that at concentrations tested, acetonitrile (1800 ppm), propionitrile (200 ppm) and isobutyronitrile (300 ppm) induced embryoletality, while fetotoxicity was observed after exposure to 200 ppm propionitrile, n-butyronitrile, or isobutyronitrile, or to 25 ppm acrylonitrile, all in the presence of overt signs of maternal toxicity. In the absence of maternal toxicity, allylnitrile (50 ppm) caused embryoletality, fetotoxicity and clear teratogenicity and n-butyronitrile (200 ppm) and methacrylonitrile (100 ppm) caused fetotoxicity. 2-chloroacrylonitrile did not cause significant embryonal or fetal toxicity at exposures up to 12 ppm, although maternal toxicity was observed. The no-observable-effect-levels for embryonal and/or fetal toxicity were established as 1500 ppm acetonitrile, 150 ppm propionitrile, 150 ppm n-butyronitrile, 100 ppm isobutyronitrile, 50 ppm methacrylonitrile, 25 ppm allylnitrile and 12 ppm acrylonitrile. It has been suggested that the degree of toxicity decreases as the aliphatic chain length increases (Johannsen 1986)<sup>4</sup>. For the saturated nitriles 2-chloroacrylonitrile was the most toxic to the pregnant rats of all the nitriles tested, but was not embryo- or fetotoxic at the highest dose tested. Acetonitrile was the least toxic to the dams and offspring, and the other three saturated nitriles were approximate in their degree of toxicity induced. However, no common toxicological profile could be drawn within the series of unsaturated nitriles tested. Although toxicity of the aliphatic nitriles is usually considered to depend upon cyanide release and upon their degree of unsaturation, under the conditions of this study, only allylnitrile exhibited teratogenic potential. Thus, it appears that other factors, in addition to cyanide liberation may play a role in the teratogenic potential of some nitriles. (Saillenfait 1993).

The effects on embryonic development was evaluated using three saturated (acetonitrile, propionitrile, n-butyronitrile) and five unsaturated (acrylonitrile, methacrylonitrile, allylnitrile, cis-2-pentenenitrile, and 2-chloroacrylonitrile) aliphatic nitriles. Using the whole embryo culture system, Day 10 rat embryos were cultured for 46 hours in the presence or absence of these materials. Each material tested produced a concentration-dependent decrease in growth and differentiation and increases in the incidences of morphologically abnormal embryos. Based on concentration, the order of increasing potency (ability to alter embryonic development) was acetonitrile < propionitrile<, n-butyronitrile, methacrylonitrile, allylnitrile < cis-2-pentenenitrile < acrylonitrile < 2-chloroacrylonitrile (the most potent). No common pattern could be drawn between the eight materials tested, although there were some similarities between malformations

elicited by the saturated nitriles. When hepatic microsomal enzymatic system was added to the culture medium it enhanced the growth retardation, dysmorphogenesis and/or lethality elicited by all five unsaturated nitriles, but had no effect on the saturated nitriles toxicity. Suggesting that potentiation of unsaturated nitriles embryotoxicity may result from generation of toxic metabolites other than or in addition to cyanide (Saillenfait 2000).

In *in vivo* experiments, rats were dose orally on Day 10 of gestation and the embryos were evaluated on Day 12 of gestation, designed to coincide with the developmental stage of the whole embryo culture system. All the nitriles investigated produced the characteristic defects developed by embryos exposed to sodium cyanide *in utero* or in culture. Further evidence that maternal production of cyanide may contribute to the developmental toxicity of nitriles (Saillenfait 2000).

References:

<sup>1</sup>Saillenfait AM and JP Sabate (2000). Comparative developmental toxicities of aliphatic nitriles: *in vivo* and *in vitro* observations. *Toxicol Appl Pharmacol.* Mar 1, 163(2):149-163.

<sup>2</sup>Saillenfait AM, Bonnet P, Guenier JP, and J de Ceaurriz (1993). Relative developmental toxicities of inhaled aliphatic mononitriles in rats. *Fundam Appl Toxicol.* Apr, 20(3):356-375.

<sup>3</sup>Willhite CC, Fern VH, and RP Smith (1981). Teratogenic effects of aliphatic nitriles. *Teratology.* June, 23(3):317-323.

<sup>4</sup>Johannsen FR and Levinskas GJ (1986). Relationships between toxicity and structure of aliphatic nitriles. *Fundam Appl Toxicol.* Nov;7(4):690-7.

**6. REPRODUCTIVE TOXICITY**

Test Substance: 2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3

No Data Found/Not Required for Closed System Intermediates under the HPV Program

This safely handled material is manufactured and transported under strict safeguards to eliminate any potential for human or environmental exposure. Conditions in which humans or the environment could be potentially exposed to 2-amino-2,3-dimethylbutanenitrile are limited and not likely to occur. As such, the reduced testing appropriate for 2-amino-2,3-dimethylbutanenitrile consists of the data already obtained: the Screening Information Data Set (SIDS) minus the tests for reproductive toxicity, developmental toxicity and chromosomal aberration. Filling these endpoints will not contribute to a greater understanding of the acute hazards to human health or the environment associated with this material and will not be of value to the safe manufacture and handling of this material

7. GENETIC TOXICITY  
a. GENE MUTATIONS<sup>1</sup>

Test Substance:	2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3
Method:	EPA OPPTS 870.5265
Type:	Salmonella typhimurium reverse mutation assay
System of testing:	TA-98, TA-100, TA-1535, and TA-1537
Concentrations:	0.1, 1, 10, 100 µg/plate (0.1 µL test substance/plate)
Controls:	Yes, Positive controls = 2-aminoanthracene (2-AA), N-methyl-N-nitro-N-nitrosoguanidine (MNNG), 9-aminoacridine (9-AA), and 2-nitrofluorene (2-NF) Negative controls = ethanol solvent control
Cytotoxic conc.:	1000, 5000 µg/plate
Metabolic activation:	with and without Aroclor induced rat liver S-9 (50 µl/plate S-9 preparation)
Year:	1983
GLP:	no
Result:	negative
Remark:	This study is assigned a reliability code of 2e according to the criteria established by Klimisch <i>et al.</i> (1997) <sup>2</sup> . It was not conducted under GLP or OECD guidelines but generally meets scientific standards, is well documented and is accepted for assessment.

Summary details:

2-amino-2,3-dimethylbutanenitrile was non-mutagenic in the Ames Salmonella Plate Assay with and without metabolic activation (S-9) using bacterial strains TA-98, TA-100, TA-1535, and TA-1537. The maximum 2-amino-2,3-dimethylbutanenitrile concentration tested was 5,000 µg/plate. 2-amino-2,3-dimethylbutanenitrile was cytotoxic at 5,000 and 1,000 µg/plate. The assay was repeated using 2-amino-2,3-dimethylbutanenitrile concentrations of 0.1, 1, 10, and 100 µg/plate. No evidence of base-pair substitution or frame-shift mutation was seen.

Strain	Substance	Concentration tested	Number of Colonies/Plate	
			Mean w/ S-9	Mean w/o S-9
TA 1535	2-amino-2,3-dimethylbutanenitrile	100	4	6
		10	10	5
		1	12	8
		0.1	10	7
		0	18	10
	2-AA	10	266	-
	MNNG	10	-	981
TA 1537	2-amino-2,3-dimethylbutanenitrile	100	4	2
		10	6	6
		1	6	9
		0.1	6	7
		0	5	6
	2-AA	10	197	-
	9-AA	50	-	575
TA 98	2-amino-2,3-dimethylbutanenitrile	100	12	6
		10	23	15
		1	24	14
		0.1	22	16
		0	22	14
	2-AA	10	1226	-
	2-NF	20	-	792
TA 100	2-amino-2,3-dimethylbutanenitrile	100	72	t
		10	70	64
		1	79	59
		0.1	96	68
		0	96	58
	2-AA	10	1705	-
	MNNG	10	-	1375

t= toxic to background bacterial lawn

References:

<sup>1</sup>Ames Bacterial/Microsome Mutagenicity Tests of CL 94,149. American Cyanamid Company, March 4, 1983.

<sup>2</sup> Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25: 1-5, 1997. See Listing of Codes, p.34.

**b. CHROMOSOMAL ABERRATIONS**

Test Substance: 2-amino-2,3-dimethylbutanenitrile, CAS# 13893-53-3

**No Data Found**

This safely handled material is manufactured and transported under strict safeguards to eliminate any potential for human or environmental exposure. Conditions in which humans or the environment could be potentially exposed to 2-amino-2,3-dimethylbutanenitrile are limited and not likely to occur. As such, the reduced testing appropriate for 2-amino-2,3-dimethylbutanenitrile consists of the data already obtained: the Screening Information Data Set (SIDS) minus the tests for reproductive toxicity, developmental toxicity and chromosomal aberration. Filling these endpoints will not contribute to a greater understanding of the acute hazards to human health or the environment associated with this material and will not be of value to the safe manufacture and handling of this material

**J. GENERAL REFERENCE**

Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25: 1-5, 1997.

1 = Valid without restriction

1a: GLP guideline study

1b: Comparable to guideline study

1c: Meets national standard methods (AFNOR/DIN)

1d: Meets generally accepted scientific standards and is described in sufficient detail

2 = Valid with restriction

2a: Guideline study without detailed documentation

2b: Guideline study with acceptable restrictions

2c: Comparable to guideline study with acceptable restrictions

2d: Meets national standard methods with acceptable restrictions

2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

2f: Accepted calculation method

2g: Data from Handbook or collection of data

3 = Invalid

3a: Documentation insufficient for assessment

3b: Significant methodological deficiencies

3c: Unsuitable test system

4 = Not assignable

4a: Abstract

4b: Secondary literature

4c: Original reference not yet available

4d: Original reference in foreign language

4e: Documentation insufficient for assessment.